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Selected Abstracts
on

**DIOXINS AND DIBENZOFURANS IN
CARCINOGENESIS, 1980-1986**

William F. Greenlee, Ph.D.
Consulting Reviewer

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of the National Cancer Institute

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INTRODUCTION

This ONCOLOGY OVERVIEW on dioxins and dibenzofurans in carcinogenesis includes abstracts selected from the CANCERLIT database referencing articles published mainly from 1980 to mid-1986. The last CANCERLIT update searched for this ONCOLOGY OVERVIEW was June, 1986.

THE STRATEGY USED TO RETRIEVE THESE ABSTRACTS CAN BE REQUESTED FROM THE CIDAC-CCB AND ENTERED ON ANY MEDLARS/MEDLINE TERMINAL SYSTEM TO UPDATE REFERENCES IN THIS AREA.

This OVERVIEW includes all aspects of the metabolism, toxicity, carcinogenicity and tumor promoting activity of dioxins and dibenzofurans, as well as the effects of these agents on enzyme induction, especially those involved in the metabolism of chemical carcinogens. The role of the binding of dioxins and dibenzofurans to specific receptors, their effects on chromosomes, nucleic acids and gene regulation, and modification of their effects are also included. In addition, their environmental occurrence, the possibility of their control through biological degradation by microorganisms, and the effect of human exposure are presented. This OVERVIEW serves to update a previous OVERVIEW, entitled "Dioxins and Dibenzofurans in Carcinogenesis" ICRDB No. OK-82/05, which included studies published mainly prior to 1980.

This Overview was prepared by Anthony J. Girardi, Ph.D., Subject Specialist for the CIDAC for Carcinogenesis and Cancer Biology, Information Ventures, Inc., Philadelphia, PA. William F. Greenlee, Ph.D. of the Department of Cell Biology, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina was the Consulting Reviewer and wrote the Editorial Commentary.

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EDITORIAL COMMENTARY

Chlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are members of a larger group of halogenated aromatic compounds (HAC) which include the biphenyls and biphenylenes, azo- and azoxybenzenes, and naphthalenes (1-3). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most potent HAC isomer and is considered the prototype for these compounds. Studies in various animal models indicate that TCDD and its isosteric analogs produce characteristic patterns of toxic and biochemical responses, separable into two major categories: those which are widely expressed (e.g., wasting, teratogenesis and thymic atrophy), and species-specific responses such as hepatotoxicity, edema of the pericardium, hyperkeratosis and chloracne (1). Acute lethality is observed at higher doses; however, there is a marked difference in species sensitivity for this response. The LD50 values (dose resulting in lethality in 50% of the treated animals) range from 2 ug/kg (guinea pig) to > 3000 ug/kg (hamster) (4).

Environmental Occurrence and Human Exposure. PCDD and PCDF isomers occur as unwanted contaminants in chlorinated phenols, in synthetic chlorophenol derivatives such as the phenoxy herbicides and hexachlorophene, and have been detected in fly ash samples from commercial incinerators (5,6). Toxic PCDF isomers also are present in overheated polychlorinated biphenyl (PCB) mixtures (7). Thus, human exposure to HACs can result from several sources including industrial accidents, chemical wastes, widespread use of chlorophenol wood preservatives and certain phenoxy herbicides, and from unforeseen sources such as the ingestion of cooking oil contaminated with PCBs and PCDFs (8). Analysis of human tissue samples of individuals without a definable source of exposure indicated the presence of part per trillion (ppt) levels of several PCDD and PCDF isomers in adipose tissue and mother's milk (5). These findings illustrate the extent of the environmental dispersion of these compounds. It was suggested that the source of one PCDF isomer, 1,2,3,7,8-pentachlorodibenzofuran, may have occurred from waste combustion (5).

Receptor-Mediated Actions. The available data from animal models and human and animal epithelial cells in culture indicate that many of the actions of TCDD on target cells are mediated by a specific receptor protein (designated the Ah receptor) (3,9). Initial studies on the subcellular distribution of the Ah receptor indicate that in the absence of ligand binding this protein is distributed predominantly within the cytosolic compartment (10). These findings are supported by the apparent cytosol-to-nucleus translocation of the TCDD-Ah receptor complex (11) and the codistribution of unoccupied receptor with cytosolic marker enzymes during fractionation (12). Volume-dilution analysis of occupied and unoccupied receptor distribution suggests that both forms reside within the nucleus and that the binding of ligand to the receptor enhances the affinity of this protein for nuclear acceptor sites (13).

Binding of the TCDD-Ah receptor complex to chromatin results in the de novo synthesis of several inducible enzyme proteins, including cytochrome P1-450 (1,3). Studies on the expression of cytochrome P1-450 messenger RNA in liver from inbred mice (14) and in murine hepatoma cell lines (15) indicate that the amount of TCDD-receptor complex in the nucleus correlates with the quantity of messenger RNA transcribed. Molecular analysis of the upstream cytochrome P1-450 regulatory genomic region isolated from a high activity murine hepatoma variant (HAV) clone has demonstrated the presence of several cis-acting control elements: a promoter, an inhibitory domain which blocks promoter function, and at least two TCDD-responsive domains (16-18). The TCDD-responsive domain from HAV

murine hepatoma cells has the properties of a transcriptional enhancer and maintains responsiveness to TCDD when transfected into either heterologous mouse tissue or a human mammary epithelial cell line (19).

The Ah receptor appears to mediate several of the toxic responses to TCDD that have been observed in animals including thymic atrophy, altered hemopoiesis, cleft palate, epidermal hyperplasia, and promotion of skin papillomas (see below) (3). Genetic analysis of TCDD-induced thymic atrophy in inbred mice indicates that this response segregates with the Ah locus (the putative structural gene for the Ah receptor), whereas TCDD-induced epidermal hyperplasia requires interaction between at least two regulatory genes, Ah and hr (20,21). The involvement of multiple regulatory genes in the expression of toxic responses in target organs other than skin and in other animal species has not been determined.

Toxicity in Humans. A number of clinical abnormalities have been reported in individuals exposed to HAPs. These include weight loss, impaired liver function, hepatic porphyria, general malaise, and chloracne (8). A recent study of 154 individuals potentially exposed to TCDD-contaminated soil (39 to 1100 parts per billion in composite samples) indicated no significant differences in several clinical parameters between exposed and control groups (22). The data suggested possible effects on cell-mediated immune function, however, no excess in clinical illness was observed (22).

Chloracne is one of the most commonly observed toxic responses to TCDD and was first reported in workers exposed to certain tars during the manufacture of hydrochloric acid (23,24). Sequential skin biopsies of individuals exposed to chloracneogens indicate that the acinar base cells of the sebaceous glands are reprogrammed to differentiate into keratinocytes (25). Plugging of the follicular orifice occurs simultaneously with the sebaceous gland duct hyperkeratinization, ultimately resulting in the formation of keratin cysts or comedones. These lesions can be accompanied by interfollicular hyperplasia (acanthosis) and hyperkeratinization (8,26).

Evidence for the presence of the Ah locus (or the regulatory gene loci equivalent to the murine Ah locus) in human cells was suggested by studies on the induction of cytochrome P1-450-mediated enzyme activities in human lymphocytes and monocytes in culture. Direct demonstration of a TCDD receptor protein has been reported in human lymphocytes, squamous cell carcinoma lines, and lung (27-29). Studies of the actions of TCDD on normal and transformed human keratinocytes in culture suggest involvement of the Ah receptor in the modulation of altered growth responses analogous to TCDD-induced hyperkeratinization *in vivo* (9).

Carcinogenicity. Chronic feeding studies in various animal species indicate that TCDD is a potent carcinogen (see Section II). In what is now regarded as a hallmark study, the carcinogenic potential of lifetime doses of TCDD ranging from 0.001 to 0.1 ug/kg/d was assessed in male and female rats (30). At a dose of 0.1 ug/kg/d, approximately one-half of the female rats developed either hepatocellular carcinoma, stratified squamous cell carcinoma of the hard palate and nasal turbinates, or keratinizing squamous cell carcinoma of the lung. A reduced incidence of tumors of the pituitary, uterus, mammary glands, pancreas and adrenal glands was observed compared with controls. At the same dose, no significant increase in the incidence of tumors was observed in male rats except for stratified squamous cell carcinomas of the hard palate, nasal turbinates, or tongue.

The genotoxic potential of TCDD has been the subject of controversy. Early studies suggested that TCDD was a direct acting mutagen for *Salmonella typhimurium* (31,32) and *Escherichia coli* (32) but, these results have not been confirmed in subsequent investigations (33,34). There is considerable evidence, including the lack of significant covalent binding to DNA (approximately 1 molecule TCDD per DNA equivalent to 35 cells), and recent demonstrations of tumor promoting actions in rats and mice (see below), that the carcinogenic potential of this compound is not the result of direct genotoxic events (35-37).

TCDD acts as a tumor promoter i.e. a two-stage model of liver carcinogenesis in the rat (36) and when painted on the skin of HRS/J hairless mice that had been previously initiated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (37). In vitro, TCDD promotes the formation of transformed foci in MNNG-initiated C3H10T1/2 cells (38). Characterization of the promoting actions of TCDD in these models suggest involvement of the Ah receptor. Specific events mediated by the interaction of TCDD with its cognate receptor relevant to the promotional response remain to be elucidated.

Several epidemiologic investigations have attempted to assess the human carcinogenic potential of TCDD. In most cases interpretation of the results is complicated by the relatively small size of the cohorts investigated. A mortality study of workers exposed to TCDD found no significant increase in the overall incidence of deaths due to cancer (39). A study of Swedish lumber workers exposed to chlorophenols and a phenoxy herbicide suggested an increased incidence of soft tissue sarcomas (40), whereas, analysis of the data from herbicide sprayers in New Zealand supported the conclusion that the phenoxy herbicide as used did not cause soft tissue sarcomas (41). Controversies have arisen concerning the diagnosis of soft tissue sarcomas (42) and, in the case of American workers exposed to TCDD, the methods used for epidemiologic analysis (43). Thus, although TCDD is a potent carcinogen (and tumor promoter) in animals, the available human epidemiologic data are inconclusive.

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ABSTRACTS

I.

CARCINOGENIC AND TOXICOLOGIC EFFECTS IN HUMANS EXPOSED TO DIOXINS AND DIBENZOFURANS

1. HEALTH EFFECTS OF LONG-TERM EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

Hoffman RE, Suter Green PA, Webb KB, Evans RG, Knutson AP, Schramm WF, Stashak JL, Gibson BR, Sorenberg KK
JAMA. 255(11):2051-2058 1986

In 1971, sludge wastes contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin were mixed with waste oil and sprayed on a dirt road at the Quail Run Mobile Home Park in Gray Summit, Mo. We performed a comprehensive examination of 154 exposed and 155 unexposed persons in the area. There were no consistent differences between the two groups on medical history, physical examination, serum and urinary chemistry studies, and neurologic tests. Results of liver function tests suggested possible subclinical effects. The exposed group had an increased frequency of anergy (11.8% vs 1.1%) and relative anergy (35.3% vs 11.8%). The exposed group also had non-statistically significant increased frequencies of abnormal T-cell subset test results (10.4% vs 6.8%), a T4/T8 ratio of less than 1.0 (8.1% vs 6.4%), and an abnormality in the functional T-cell test results (14.6% vs 8.5%). These findings suggest that long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin is associated with depressed cell-mediated immunity, although the effects have not resulted in an excess of clinical illness in the exposed group. Further studies are indicated to elucidate the pathophysiology and clinical significance of these immunologic findings.

2. SOFT TISSUE SARCOMA RISK IN SWEDISH AGRICULTURAL AND FORESTRY WORKERS

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JNCI. 76(2):229-34 1986

The risk of soft tissue sarcoma following possible exposure to phenoxy acid herbicides was studied in 354,620 Swedish men, who were employed in agriculture or forestry according to a national census in 1960. This cohort was further divided into six subcohorts, on assumed exposure to phenoxy acid herbicides. The most commonly used phenoxy acid in Sweden was (4-chloro-2-methylphenoxy)acetic acid (CAS: 94-74-6). The reference cohort encompassed 1,725,845 Swedish men employed in other industries. All persons were followed up in the cancer-environment register during the period 1961-79. A total of 331 cases of soft tissue sarcoma was observed in the study cohort and there were 1,308 cases in the reference group [relative risk (RR), 0.9; 95% confidence interval, 0.8-1.0]. No subcohort of agricultural or forestry workers showed any significantly increased RR, nor was there any significant difference in RR between the subcohorts. Despite the greatly increased use of phenoxy acid herbicides from 1947 to 1970, no time-related increase in the RR of soft tissue sarcoma was found in the total cohort or in any of the subcohorts.

3. NON HODGKIN'S LYMPHOMA AND EXPOSURE TO PHENOXYHERBICIDES, CHLOROPHENOLS, FENCING WORK, AND MEAT WORKS EMPLOYMENT: A CASE-CONTROL STUDY

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Department of Community Health, Wellington Clinical School of Medicine, Wellington Hospital, New Zealand
Br J Ind Med. 43(2):75-85 1986

A previous case-control study which used the occupational information available on the New Zealand Cancer Registry found that agricultural workers were at increased risk of developing non-Hodgkin's lymphoma. The findings are now presented for the second phase of the study which entailed interviewing 85 cases of non-Hodgkin's lymphoma registered under code 202 of the International Classification of Diseases together with 160 controls with other types of cancer and 228 general population controls. The findings for the two control groups were similar, and there were no significant differences between cases and controls regarding potential exposure to phenoxy-herbicides (odds ratio = 1.4, 90% confidence limits 0.7-2.5, $p = 0.26$) or chlorophenols (odds ratio = 1.3, 90% confidence limits 0.6-2.7, $p = 0.39$). The odds ratio for fencing work, necessitating exposure to several potential risk factors including arsenic and sodium pentachlorophenolate was 2.0 (90% confidence limits 1.3-3.0, $p = 0.01$). The odds ratio for employment in a meat works, necessitating potential exposure to 2, 4, 6-trichlorophenol and acetoxy viruses, was 1.8 (90% confidence limits 1.1-3.1, $p = 0.04$). There was a significant statistical interaction between the risks associated with these two activities, the odds ratio for involvement in both activities compared with involvement in neither being 5.7 (90% confidence limits 2.3-14.3, $p = 0.03$).

4. CORRELATION OF PLACENTAL MICROSOMAL ACTIVITIES WITH PROTEIN DETECTED BY ANTIBODIES TO RABBIT CYTOCHROME P-450 ISOZYME 6 IN PREPARATIONS FROM HUMANS EXPOSED TO POLYCHLORINATED BIPHENYLS, QUATER PHENYLS, AND DIBENZOFURANS

Wong TK, Duman BA, Best PE, Blanton TL, Anderson MW, Philpott RM

Biochemical Risk Analysis Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709
Cancer Res. 46(21):990-1004 1986

Placental tissues were obtained from Chinese women in Taiwan who had been exposed to contaminated rice oils containing polychlorinated biphenyls and their thermal degradative products. Exposure via the diet occurred 4-5 years prior to pregnancy. Placental microsomal fractions from eight of the nine exposed subjects studied showed marked elevation of benzo(a)pyrene hydroxylation and 7-ethoxycoumarin O-deethylation activities related to control subjects. Placental microsomes from exposed subjects were found to contain a protein that cross-reacted with antibodies raised to rabbit cytochrome P-450 isozyme 6, an isozyme induced by polycyclic aromatic hydrocarbons. This protein was not observed with microsomal samples from control subjects. A significant correlation was found between the relative amounts of the im-

monoreactive protein and benzo(a)pyrene hydroxylation and 7-ethoxycoumarin O-deethylase activities. The 7-ethoxycoumarin O-deethylase activities were inhibited by alpha-naphthoflavone, a compound known to inhibit activities of rabbit cytochrome P-450, isozyme 6.

5. IS 2,3,7,8-TCDD (DIOXIN) A CARCINOGEN FOR HUMANS?

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Environ Health Perspect, 62:329-33 (1985)

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has suddenly become the focal point of controversy over the relationship of chemical waste to human health. Specific concern exists regarding its potential association with human malignancy. Subcellular, cellular, and whole-animal experiments suggest that TCDD exerts much of its activity by inducing enzymes that protect the intact organism from the assault of environmental contamination. TCDD is a potent inducer of aryl hydrocarbon hydroxylase, although wide variations between species do exist. Conventional tests for mutagenicity have produced conflicting results. Animal experiments have shown the development of tumors following chronic low level ingestion of TCDD. The human evidence regarding the potential carcinogenicity of TCDD comes from occupational, military and environmental exposures. Several studies have come out of Sweden suggesting an association between sarcoma and exposure to herbicides. Although there is little solid evidence that 2,3,7,8-TCDD produces substantial chronic disability or premature death in man, a significant body of experimental evidence for its carcinogenicity makes it likely that a small number of human malignancies may be due to its action. Since 2,3,7,8-TCDD is an unwanted contaminant it could be eliminated with little measurable consequence.

6. COMMENTARY ON 2,3,7,8-TETRACHLORODIBENZO-PARA-DIOXIN (TCDD)

Molrison H, Baker C, Kimber R, Lawrence R, Loney F,

Winters W, Carraro TR, Handel LK, Rumsch BH, Sypher D,

Vet Hum Toxicol, 27(5):444-8 (1985)

There is deep concern about the long term health effects of exposure to phenoxy herbicides and the contaminant TCDD; however, there is considerable scientific and medical uncertainty regarding the health effects from exposure to these chemicals. There are at least ten ongoing studies on reproduction, morbidity and mortality as well as studies of tissue concentrations of TCDD that are attempting to determine the health effects of these chemicals (see Table 2). Appropriate efforts should be made to prevent human and environmental exposure and to decontaminate the environment while awaiting the results of these investigations. Animal toxicity studies show wide variations that extrapolations from a different species to humans are tenuous. Human studies on exposed workers and nonoccupational exposures are difficult to interpret because the exposure has not been quantified and because workers were exposed to mixtures of chemicals. Chloracne appears to be an important specific clinical marker of TCDD exposure, however, it can be caused by structurally similar compounds. Many of the past studies on human health effects of 2,4,5-T and TCDD are controversial. Since the scientific data are not firm, no specific statements can be made regarding the long term health effects at this time. Any individual who has had a significant exposure to TCDD should see his/her physician and have appropriate consultation. Long term follow up will be required. Physicians should be instructed regarding the possible manifestations of TCDD exposure to look for chloracne, soft tissue

masses, muscle pain, fatigue, peripheral neuropathy, tender hepatic enlargement, enlargement, elevated liver enzymes, elevated lipids, prolonged prothrombin time, hemorrhagic cystitis and hirsutism. (ABSTRACT TRUNCATED AT 250 WORDS)

7. EVALUATION OF CHROMOSOMAL DAMAGE IN MALES EXPOSED TO AGENT ORANGE AND THEIR FAMILIES

Kaye CI, Rao S, Singam SJ, Rosenthal PS, Cohen MM

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J Chromatogr Genet Dis Biol Suppl, 1:299-63 (1985)

Agent Orange (AO), a phenoxyherbicide, and dioxin, an impurity found in AO, are considered clastogens, mutagens, and teratogens in plants and animals. AO has come under suspicion in humans following claims that it causes chromosome damage and birth defects in offspring of exposed individuals. No well-designed epidemiological studies are available to support this conclusion. Of ten exposed individuals studied for chromosome breaks and sister chromatid exchange frequencies, eight were ascertained because they had children with congenital defects. No consistent pattern of anomalies was observed. Five children had neurologic deficit, one child had a central nervous system anomaly, and one child was affected with glaucoma. Although all individuals studied had normal karyotypes, a statistically significant increase in chromosome breakage was observed in exposed males compared to their unexposed wives and children; sister chromatid exchange frequency was not increased.

8. POTENT INDUCTION OF HUMAN PLACENTAL MONO-OXYGENASE ACTIVITY BY PREVIOUS DIETARY EXPOSURE TO POLYCHLORINATED BIPHENYLS AND THEIR THERMAL DEGRADATION PRODUCTS

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Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Environ Health Perspect, 62:221-4 (1985)

Placental tissues were obtained from women in Taiwan who had ingested rice oil contaminated with aromatic polychlorinated hydrocarbons 3-4 years before conception. Known contaminants in the rice oil were polychlorinated biphenyls and their thermal degradation products, including polychlorinated dibenzofurans and polychlorinated quaterphenyls. Placental homogenates showed large increases in mono-oxygenase enzymes, including aryl hydrocarbon hydroxylase, 7-ethoxycoumarin O-deethylase, and diol, quinone, and phenolic metabolites of benzo(a)pyrene (analysed by high performance liquid chromatography). The findings suggest that exposure to these widespread, environmental pollutants may exert substantial and persistent metabolic effects.

9. CHLORACNE AND CHLORACNEGENS

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J Am Acad Dermatol, 13(4):595-98 (1985)

Chloracne, an acneform eruption resulting from poisoning by halogenated aromatic compounds, has been a considerable problem over the last 40 years. The condition is always a symptom of systemic poisoning and should be familiar to all practitioners, particularly dermatologists. It is difficult to treat and can last for long periods without known additional exposure to chloracne. Some chloracne is capable of causing a variety of systemic signs and symptoms and may be oncogenic. Although there are probably fewer than 4,000 persons with chloracne worldwide, those found with the dis-

order should be evaluated medically on a regular basis and followed, if possible, throughout their lives. (85 Refs)

10. CHLORACNE, THE HALLMARK OF DIOXIN INTOXICATION*

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Scand J Work Environ Health; 11(3 Sup No):165-71 1985

Clinical experiences and laboratory studies are described involving a population of workers who were exposed in a plant making 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), including a trichlorophenol runaway reaction. Workers were followed for a period of four years, and 30 years later a mortality analysis was done on those exposed to runaway reaction material to determine possible increased risks for causes of death. Subsequently, a morbidity study on 436 employees involving three cohorts was carried out to determine the long-term health effects associated with the production of 2,4,5-T including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The mortality and morbidity studies demonstrated that the standardized mortality ratio for all causes of death was 69, and for cancer at all sites and cardiovascular disease it was 100 and 68, respectively. The most significant observations emerging from the morbidity study were that 86% of the exposed persons developed chloracne at some time and that 52.7% still had chloracne on examination 20 to 30 years after the initial exposure. There appeared to be no evidence, on a long-term basis, of increased risks for: cardiovascular disease, hepatic disease, renal disease, central and peripheral nerve problems, reproductive problems, or birth defects among the exposed and those who had chloracne among the exposed. Studies on the cell kinetics and pathogenesis of chloracne indicate that TCDD induces the modulation of undifferentiated sebaceous gland cells to xeroderma. This action results in a disappearance of sebaceous glands and substitution of closed comedones and keratin cysts. Production workers have the highest frequency and severity of chloracne and systemic effects. (ABSTRACT TRUNCATED AT 250 WORDS)

11. A FOLLOW-UP STUDY OF CANCER INCIDENCE AMONG WORKERS IN MANUFACTURE OF PHENOXY HERBICIDES IN DENMARK

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Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark

Br J Cancer; 52(2):279-70 1985

The purpose of this cohort study is to shed further light on the potential carcinogenic effect indicated by a Swedish case control study of the 2,4-dichlorophenol and 4-chloro-ortho-cresol based phenoxy herbicides, unlikely to be contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). In the present study it was the intention to include all persons employed in manufacture of phenoxy herbicides in Denmark before 1982. The predominant product was MCPA and only a very limited amount of 2,4,5-T was processed in one of the two factories included in the study. Registration of the cohort was based on company records, supplemented with data from a public pension scheme from 1964 onwards. Ninety-nine percent of registered employees could be followed up. Cancer cases were identified by linkage with the National Cancer Register. Totals of 3,390 males and 1,069 females were included in the study. In the analysis special attention was given to soft tissue sarcomas (STS) and malignant lymphomas (ML) which are the diagnostic groups indicated to be associated with exposure to phenoxy herbicides in the Swedish studies. Five cases of STS were observed among male employees in contrast to 1.84 expected cases.

This result supports the Swedish observation of an increased risk of STS following exposure to phenoxy herbicides unlikely to be contaminated with 2,3,7,8-TCDD. However, several potential biases have to be taken into account in interpretation of this observation and these are discussed. Seven cases of ML were observed among male employees in contrast to 5.37 expected which does not support the Swedish observation of an excess risk. The total cancer risk among persons employed in manufacture and packaging of phenoxy herbicides was equivalent to the cancer risk in the Danish population. Among males thus employed 11 lung cancer cases were observed in contrast to 5.33 expected. Attention should be given to exposure to spray dried MCPA-sodium salt in the plants, but other work place exposures and tobacco consumption may have contributed to the increased risk. The tabulation of data by many diagnostic groups may explain the excesses observed for rectum cancer among males and cervical cancer among females. The study has revealed that several potential biases have to be taken into account when the Swedish observations are tested in other settings.

12. CHLOROPHENOL EXPOSURE IN SAWMILLS

Kauppinen T, Lindroos L

Institute of Occupational Health, Helsinki, Finland

Am Ind Hyg Assoc J; 46(1):34-8 1985

Personnel exposures to chlorophenols were measured in ten Finnish sawmills where a chlorophenolate salt formulation was used for the blue stain control of sawed wood. The concentrations of chlorophenols in the air were usually well below the occupational limit value of 0.5 mg/m³. However, high concentrations (above 10 mg/m³) were measured inside heated kilns. Additionally, high concentrations were detected in the urine of workers whose skin came into contact with chlorophenol solution, indicating that chlorophenols are rapidly absorbed through the skin. The concentrations of chlorophenols in the air were low in trimming-grading plants where treated boards are handled, but some of the chlorophenols was bound to wood dust which is deposited mainly in the nose. The risks associated with the impurities contained in chlorophenol preparations (chlorinated dibenzodioxins and dibenzofurans) and the need for epidemiologic studies on possible cancer risks in sawmill work are discussed.

13. COMMENTARY ON 2,3,7,8-TETRACHLORODIBENZO-PARA-DIOXIN (TCDD). SCIENTIFIC REVIEW COMMITTEE OF THE AMERICAN ACADEMY OF CLINICAL TOXICOLOGY

Anonymous

J Toxicol Clin Toxicol; 23(2-3):191-204 1985

Keywords (MeSH): Animal; Chemistry; Dioxins/*toxicity; Female; Human; Kinetics; Pregnancy; Reproduction/drug effects; Sarcoma/chemically induced; Skin/drug effects; Soft Tissue Neoplasms/chemically induced; Tetrachlorodibenzo-dioxin/metabolism/*toxicity.

14. OCCUPATIONAL EXPOSURES ASSOCIATED WITH MALE REPRODUCTIVE DYSFUNCTION (172 Refs)

Schnag SD, Dixon RL

Office of Health Research, US Environmental Protection Agency,

Research Triangle Park, North Carolina 27711

Ann Rev Pharmacol Toxicol; 25:567-92 1985

Keywords (MeSH): Anesthetics/adverse effects; Arsenic/adverse effects; Benzene/adverse effects; Boron/adverse effects; Cadmium/adverse effects; Calcium Disulfide/adverse effects; Contraceptives, Oral; Hormonal/adverse effects; Dinitrobenzenes/adverse effects; Ethylene Dibromides/adverse effects; Human; Infertility, Male/*chemically induced; Kepone/adverse effects; Lead/adverse effects; Male; Mangan-

new/adverse effects; Methylmercury Compounds/adverse effects; Occupational Diseases/*chemically induced: Pentachlorophenol/adverse effects; Pesticides/adverse effects; Propane/analogues and derivatives/adverse effects; Reproduction/drug effects; Review; Sevin/adverse effects; Solvents/adverse effects; Tetrachlorodibenzo-dioxin/adverse effects; Vinyl Chloride/adverse effects.

15. ARE OFFSPRING AT RISK FROM THEIR FATHER'S EXPOSURE TO TOXINS? [NEWS]

Brown NA

Nature. 316/6024:110 1983

Keywords (MeSH): Abnormalities, Drug-Induced/*familial and genetic; Abnormalities, Radiation-Induced/*familial and genetic; Animal; Cyclophosphamide/toxicity; Ethylnitrosourea/toxicity; Fathers; Human; Male; Mice; Mutation; Rats; Spermatocyst/drug effects/radiation effects; Tetrachlorodibenzo-dioxin/adverse effects; 2,4-Dichlorophenoxyacetic Acid/adverse effects; 2,4,5-Trichlorophenoxyacetic Acid/adverse effects.

16. THE AGENT ORANGE CONTROVERSY IN AUSTRALIA: A CONTRIBUTION TO THE DEBATE

Hall W, MacPhail D

Community Health Stud. 9(2):109-19 1983

Keywords (MeSH): Abnormalities, Drug-Induced/etiology; Australia; Dioxins/*adverse effects; Human; Neoplasms/chemically induced; Organic Mental Disorders, Substance-Induced/etiology; Tetrachlorodibenzo-dioxin/*adverse effects; *Veterans; Vietnam; 2,4-Dichlorophenoxyacetic Acid/*adverse effects; 2,4,5-Trichlorophenoxyacetic Acid/*adverse effects.

17. DIOXIN EXPOSURE AT MONSANTO [LETTER]

Gough M

Nature. 316/6046:304 1983

Keywords (MeSH): *Dioxins, Environmental Exposure; Heart Diseases/chemically induced; Human; Neoplasms/chemically induced; Occupational Diseases/*chemically induced; West Virginia.

18. AN EVALUATION OF REPORTS OF DIOXIN EXPOSURE AND SOFT TISSUE SARCOMA PATHOLOGY AMONG CHEMICAL WORKERS IN THE UNITED STATES

Fingert MA, Halpern WE, Hancher PA, Smith AB, Gough DH, Russell WO

National Institute for Occupational Safety and Health, Cincinnati, Ohio

Scand J Work Environ Health. 10(5):299-303 1984

A review of employment records and tissue specimens of seven workers, reported previously as having occupational dioxin exposure and soft tissue sarcomas, confirms that four workers had employment of 2 to 19 years in the production of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) or trichlorophenol, products contaminated with 2,3,7,8-tetrachlorodibenzo-dioxin, the most toxic dioxin isomer. Of these individuals, two have confirmed soft tissue sarcomas. In addition three individuals who worked for companies which made 2,4,5-T also have confirmed soft tissue sarcomas. Their employment records do not show specific assignment to 2,4,5-T or trichlorophenol departments; however, one individual worked for 10 d in the production of pentachlorophenol, which is contaminated with different isomers of dioxin. Methodological problems are discussed which must be addressed in the epidemiologic evaluation of the outcome of soft tissue sarcoma.

19. SARCOMAS OF SOFT TISSUES AFTER VIETNAM SERVICE

Greenwald P, Kimmissey B, Collins DN, Thurnwald G
Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, Md

JNCI. 73(3):1107-9 1984

Vietnam service and military service experiences of 281 men with sarcomas of soft tissues were compared in this epidemiologic study to a control group of men derived from driver's license files and matched on 5-year period of birth and ZIP code of residence. No direct association was found for service in Vietnam (odds ratio, 0.53; confidence limits, 0.21-1.31) or for any military service (odds ratio, 0.53; confidence limits, 0.37-0.76). A multivariate matched logistic regression analysis showed similar results for Vietnam service while controlling for military service. Results also were similar when the 130 cases who had died were compared to a second control group derived from death certificates. Finally, no significant associations were found for 'Agent Orange' or other variables that might be related to herbicide exposure.

20. SOFT TISSUE SARCOMA AND EXPOSURE TO PHENOXYHERBICIDES AND CHLOROPHENOLS IN NEW ZEALAND

Smith AH, Pearce NE, Fisher DO, Giles HJ, Tappin CA, Howard JK

Department of Community Health, Wellington Clinical School of Medicine, Wellington Hospital, New Zealand

JNCI. 73(3):1111-7 1984

Phenoxyherbicides, including (2,4,5-trichlorophenoxy)acetic acid (CAS: 93-76-5), have been widely used in New Zealand for over 30 years. In the light of Swedish studies reporting an association between exposure to phenoxyherbicides or chlorophenols and soft tissue sarcoma, a case-control study was undertaken that involved interviewing 82 subjects (cases) with soft tissue sarcoma and 92 controls with other types of cancer. For those potentially exposed to phenoxyherbicides for more than 1 day not in the 5 years before cancer registration, the estimate of relative risk was 1.3, with 90% confidence limits of 0.6-2.5. The comparable relative risk estimate for chlorophenol exposure was 1.5, with 90% confidence limits of 0.5-4.5. The discovery of cases in trichlorophenol manufacturing plants in the United States lent support to the Swedish findings, but further studies are needed to conclude whether human exposure to these chemicals truly increases the risk of soft tissue sarcoma.

21. TOXIC EFFECTS OF IN VIVO DIOXIN-CHLOROPHENOL EXPOSURE ON HUMAN LEUCOCYTE FUNCTIONS

Prasse JK

University of Illinois at Chicago, Health Sciences Center, Div. Allergy Immunol. 43(7):2111B 1984

Membrane related leukocyte functions were examined in peripheral circulating blood lymphocytes and neutrophils from 47 men exposed to a dioxin contaminated batch of chlorophenols. Prior analysis showed the dioxin present in blood samples. Peripheral blood samples were drawn by venipuncture. Routine hematology and differentials were performed on all samples. Lymphocytes and neutrophils were isolated on Ficoll-Hypaque density gradient, washed and suspended in nutrient medium, counted and analyzed in vitro. ^3H -Thy labelled DNA was measured in lymphocytes stimulated by PHA, PWM, Con-A mitogens. Subject percentages were determined via spontaneous bacterial binding technique. Opsonized zymosan or IgG coated latex biobeads were used to induce phagocytosis and respiratory burst activity measuring luminol amplified chemiluminescence of oxygen reduction.

Nitroblue tetrazolium assay also examined phagocytic activity visually. Neutrophil cytosolic ATP was also measured by chemiluminescence. Patients and controls were age matched and resulting data was evaluated clinically and statistically using Welch's T test, Fisher's F test and the Mahalanobis-Hoelling simultaneous multivariate analysis model. The results of this study demonstrated clinically significant enhanced alteration of B-cell proliferative response ($p < 0.001$) and increased T-cell responsiveness, as well as altered ratios of T-cells (expanded) to B-cell (reduced) subsets ($p < 0.001$). Further, neutrophil chemiluminescence was significantly depressed (p is less than 0.01) indicating reduced killing activity, confirmed by the NBT assay. Neutrophil cytosolic ATP levels were also reduced (p is less than 0.001). Hematological analyses demonstrated frequent erythrocytic anisocytosis, slight lymphocytosis, neutropenia and a reverse d ratio of neutrophils to lymphocytes in peripheral blood. The results of this study lead to the conclusion that a large proportion of the dioxin exposed patients exhibited a loss of hematopoietic integrity as well as an impaired immune-host defense system. The loss of immune and hematopoietic systems integrity agrees well with the responses seen in experimental animal models. The loss of leukocyte integrity may result in frequent or recurring infectious challenge, autoimmune and/or malignant disease. (Author abstract)

22. DOES AGENT ORANGE CAUSE BIRTH DEFECTS?

Frederick JN

Department of Obstetrics and Gynecology, University of Texas Health Science Center, Southwestern Medical School, Dallas
Teratology. 29(2):193-221 1984

Large quantities of the defoliant, Agent Orange, were sprayed in Vietnam during the war. Agent Orange was composed of two herbicides: 2,4-D and 2,4,5-T, the latter contaminated by small amounts of a highly toxic dioxin (TCDD). The constituents of Agent Orange are capable of producing gene mutations and chromosomal aberrations, at least in some experimental circumstances. TCDD and 2,4,5-T are teratogenic in mice and perhaps in other mammals, but the teratogenicity of these chemicals has not been convincingly demonstrated in humans. There is currently no scientific evidence which indicates that men who were previously exposed to Agent Orange are at increased risk of having children with birth defects, but available data are inadequate to assess this possibility critically. (105 Refs)

23. CUTANEOUS SIGNS OF SYSTEMIC TOXICITY DUE TO DIOXINS AND RELATED CHEMICALS

Danaghe WF

Division of Dermatology, University of Missouri, Columbia
J Am Acad Dermatol. 10(4):686-700 1984

The controversy about dioxin effects on human health received a great deal of attention recently when the State of Missouri was declared to be in a dioxin crisis. However, dioxin and several related chemicals are widespread throughout the world. Cutaneous signs play an important part in evaluating toxicity of dioxin and similar chemicals. Chloracne is the most sensitive indicator of significant dioxin exposure. Porphyrria cutanea tarda and hyperpigmentation are other known cutaneous effects, and malignant fibrous histiocytomas of the skin may possibly be associated, although data are inconclusive on this point. The AMC Council on Scientific Affairs recommended that all physicians become familiar with chloracne and other toxic effects of dioxin. Dermatologists, especially, should be aware of the problem and may discover early cases of previously unsuspected exposure to this group of chemicals. (82 Refs)

24. HEALTH EFFECTS OF DIOXIN (LETTER)

Sarling TD

Science. 225(4641):1262 1984

Keywords (MeSH): *Abnormalities, Drug-Induced; Animal; Dioxins/*toxicity; Human; Infant, Newborn; Neoplasms/*chemically induced; Tetrachlorodibenzo-dioxin/*toxicity.

25. AGENT ORANGE STUDY IS LIKE A CHAMELEON

Fox JL

Science. 225(4641):1156-7 1984

Keywords (MeSH): Abnormalities, Drug-Induced/occurrence; Acute/occurrence; Dioxins/*toxicity; Human; Infant Mortality; Infant, Newborn; Liver Diseases/chemically induced/occurrence; Male; Mental Disorders/chemically induced/occurrence; *Military Medicine; Neoplasms/chemically induced/occurrence; Statistics; Tetrachlorodibenzo-dioxin/*toxicity; 2,4-Dichlorophenoxyacetic Acid/*toxicity; 2,4,5-Trichlorophenoxyacetic Acid/*toxicity.

26. AGENT ORANGE: AUSTRALIAN STUDY CONTINUES (NEWS)

Sidler J

Nature. 310(5978):534 1984

Keywords (MeSH): Australia; Dioxins/*toxicity; Human; *Military Medicine; Neoplasms/chemically induced; Tetrachlorodibenzo-dioxin/*toxicity; 2,4-Dichlorophenoxyacetic Acid/*toxicity; 2,4,5-Trichlorophenoxyacetic Acid/*toxicity.

27. MEDICAL AND MORBIDITY SURVEILLANCE FINDINGS AMONG EMPLOYEES POTENTIALLY EXPOSED TO TCDD

Bond GG, Ott NG, Browner FE, Cook RR

Michigan Division Medical, Dow Chemical USA, Midland, Michigan

Br J Ind Med. 40: 318-24 1983

Available medical and morbidity surveillance findings from 1976 to 1978 for two employee cohorts potentially exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were compared with those of matched unexposed employees. The medical surveillance findings were derived from a screening programme offered to all active employees and included an analysis of various medical history questions and blood chemistry results. Group medical insurance claims served as the source of morbidity surveillance data and the period prevalence of selected diseases was analysed. Few significant differences between the exposed and unexposed were detected. Among the cohort of employees potentially exposed during the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), a significantly greater frequency of a-ray proved ulcer was reported and significantly more members of this group had diseases of the digestive system diagnosed. Such findings were absent in the more highly TCDD-exposed cohort engaged in 2,4,5-trichlorophenol production, making it unlikely that dioxin was a cause.

28. MORTALITY OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4,5-TRICHLOROPHENOXYACETIC ACID HERBICIDE APPLICATORS IN FINLAND. FIRST REPORT OF AN ONGOING PROSPECTIVE COHORT STUDY

Riihimäki V, Aap S, Hänninen S

Inst. Occupational Health, Haartmaninkatu 1, SF-00290 Helsinki 20, Finland

Scand J Work Environ Health. 8(1):37-42 1982

Mortality and cancer incidence in a group of 1,926 males occupationally exposed to both 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid was followed over a 9-year period. The group consisted of those with 2-7 work

weeks exposure (75%) and those with greater than 8 work weeks exposure (25%) prior to the study period and not necessarily in the same year. Further exposure during the study period may have occurred. Initial exposure of two-thirds of the group occurred within 5 years of the start of the study period. The ratio of observed to expected deaths was 62/92.9 in the initial 5-year period and 82/91.1 in the final 4-year period; the corresponding figures for cancers were 9/18.1 and 17/18.4, respectively; and for all natural causes of death, 41/76.7 and 64/78.6. The same undermortality tendencies were seen allowing for 10- and 15-year latency periods. No lymphoma or soft tissue sarcoma deaths occurred in the 10-year latency period. Increased mortality or cancer was not seen in groups with exposure greater than or equal to 6 or 8 weeks in a 5-year period. Smoking habits were not questioned. (30 Refs)

29. DIOXIN EXPOSURE AND SARCOMA [LETTER]

Fragerberg MA, H. Jernin WE
JAMA. 269(23):3176-7 1983

Keywords (MeSH): Dioxins/*adverse effects; Human; Occupational Diseases/*chemically induced; Sarcoma/*chemically induced; Soft Tissue Neoplasms/*chemically induced.

30. TETRACHLORODIBENZODIOXIN: A SURVEY OF SUBJECTS TEN YEARS AFTER EXPOSURE

May G
Bulwer, Chesterfield, Derbyshire, England
Br J Ind Med. 39(2):128-135 1982

Ten years after an incident following which 79 workers developed chloracne due to exposure to tetrachlorodibenzo-dioxin a study was undertaken to establish the current state of health of the affected employees remaining in the company's (Coalite Oils and Chemicals Ltd, a subsidiary company of Coalite Group Ltd) employment. The opportunity was used to examine effects on mortality, morbidity, carcinogenesis, reproduction, teratogenicity, fetotoxicity, biochemistry, immunology, and genetic change. Concurrently, control groups were established with which to make comparison. The control groups selected from within the works matched the study group in respect of sex and age but it was not possible to match them for occupation and social status. Half the affected subjects still have minor chloracne. Other than this there is no evidence that they have been adversely affected in any way. (Author abstract) (24 Refs)

31. HEALTH EFFECTS OF AGENT ORANGE AND DIOXIN CONTAMINANTS

Dolan WD, Belzer JR, Cooper T, Friedlander IR, Gifford RW, Karian MR, Masley JH, Schmidt RT, Skow JH, Smith RJ, Snow JB, Tupper CJ, Jones RJ
(1/2 June) Council on Scientific Affairs, American Medical Association, 535 N Dearborn St., Chicago, IL 60610
JAMA. 248(15):1895-1897 1982

The medical evidence regarding the toxicity and long-term health effects of Agent Orange (AO) and the associated contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is reviewed. No clearly defined mutagenic effect was observed in vitro with TCDD. TCDD induced genetic changes by the Ames test with *Salmonella typhimurium* and *Escherichia coli*, but not with repair-defective strains. No evidence from dominant lethal and cytogenetic evaluations in rodents was obtained that genetic changes occur in whole animals. In vitro studies on HeLa cells, BALB/3T3 normal mouse fibroblasts, simian virus SV 101, SV 40-transformed 3T3 mouse fibroblasts, and normal human lymphocytes detected no significant growth inhibition in the cell cultures nor discernible ultrastructural changes. A number of studies which are under way may resolve the questions of kinds and extent of human

damage due to exposure to low levels of TCDD. It was recommended that all physicians should be alerted to the classic signs of chloracne and the possible signs and adverse effects of TCDD exposure. Persons who had serious exposures to TCDD should be identified and treated and vital information that is needed for the ongoing human epidemiologic studies should be collected. (no Refs)

32. DO PHENOXY HERBICIDES CAUSE CANCER IN MAN?

Cagan D, Acheson ED
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Southampton General Hosp., South Academic Block, Southampton
SO9 4XY, England
Lancet. 1(8280):1057-1059 1982

Two Swedish case-control studies have shown an increased risk of soft-tissue sarcomas in men exposed to phenoxy herbicides and chlorophenols during their application. A high incidence of soft-tissue sarcomas has also been observed among workers employed in the manufacture of these products in the USA. Other studies have failed to demonstrate this association. A third case-control study in Sweden suggests that phenoxy acids and chlorophenols may also predispose to Hodgkin's disease and non-Hodgkin's lymphoma, but as yet there is little support for this theory from other sources. Further research is urgently needed to confirm or refute these associations, to define the extent of the risk (if any), and to identify the carcinogen(s). (Author abstract) (31 Refs)

33. THE STUDY OF X-RAYS AND TCDD EFFECTS ON SATELLITE ASSOCIATIONS MAY SUGGEST A SIMPLE MODEL FOR APPLICATION IN ENVIRONMENTAL MUTAGENESIS

Di Lorenzo R, Crisafulli C, Puchetti G
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20133 Milan, Italy
Hum Genet. 65(1):42-47 1982

The effects of x-rays on satellite association (SA) pattern were tested and the results of SA analysis on 30 individuals (16 accidentally exposed to dioxin, TCDD) were compared. Two hundred control and 200 irradiated metaphases were compared. The relative frequency of D group chromosomes involved in SA (DSAf) was significant in the whole blood at 100 rad, 150 rad, and 200 rad in selected metaphases with chromosome aberrations. The mitotic index was measured at 100, 200, and 400 rad to evaluate cytotoxicity dependent on the x-ray dose. Numbers of SA/cell (SAc) and associated chromosomes/cell (DGc) which are correlated, decreased linearly with the dose of irradiation and in parallel with the mitotic index. A relationship was observed between chromosome damage induced by x-rays and the significant variations measured in DSAf and numbers of SAc and DGc. No significant differences in the av number of SAc and DGc between controls and samples obtained of subjects exposed to TCDD were measured. Fluctuations were definitely more noticeable in the av frequencies of D group chromosomes. Results appeared to be in accordance with the hypothesis based on random damage of functional nucleolar organizing regions. (32 Refs)

34. [PHENOXY HERBICIDES AND MYOMA]

Shibasaki K
Machibaraizuka Hsp., Japan
Kansai. 57(1):79 1982

Swedish and American studies on the occurrence of soft tissue myomas and malignant lymphomas in humans exposed to phenoxy herbicides are reviewed. Use of such herbicides in household gardens in Japan suggests that such tumors could occur in Japanese. The mixing of tetrachlorodibenzo-

dioxin in waters of the sewers, lakes, and marshes of Japan, due to use of the herbicides in forestry and agricultural industries, also makes a high potential for the occurrence of soft tissue tumors in Japanese. (no Refs)

35. CONSIDERATIONS ON THE EPIDEMIOLOGY OF THE SEVESO ACCIDENT

Donatelli L, Lampa E, Cusi E

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Toxicol. Eur. Rev. 3(1):9-16 1981

A historical analysis is presented of the accident at an ICMESA plant in Meda, Italy, in 1976. The accident resulted in the release of a toxic cloud into the atmosphere that affected Seveso and other areas to the southeast. A critical evaluation of the investigations conducted up to 1980 is presented. The reactor involved was used for the synthesis of 2,4,5-trichlorophenol (TCP), and the toxic cloud contained TCP, 2,4,5-sodium trichlorophenolate, 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD), predioxins, ethylene glycol, sodium hydrosulfide, and possibly, tetrachlorobenzene residues. The accident is discussed with respect to dioxin and combinations which may alter its toxicity, toxicity and animal species, severity indices in the Seveso accident, and evidence and views concerning the dangers and problems associated with the accident. It was concluded that quantitative evaluations must be used to determine the true consequences of dioxin contamination of the area, and that new laws are urgently needed to protect people working and living in toxic environments. The data collected up to now on mutagenicity and carcinogenicity fail to suggest an effect of the exposure at Seveso in man, and does not permit prediction of future danger. (37 Refs)

36. SOFT-TISSUE SARCOMAS AND EXPOSURE TO CHEMICAL SUBSTANCES: A CASE-REFERENT STUDY

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Br J Ind. Med. 38(1):27-33 1981

In 1977 several patients were seen with soft-tissue sarcomas and previous exposure to phenoxy acids. This clinical observation resulted in a case-referent (case-control) study being undertaken which showed that exposure to phenoxy acids or chlorophenols, which are chemically related, gave a roughly six-fold increase in the risk for this type of tumor. A further case-referent study of soft-tissue sarcomas has now been performed to confirm these earlier findings and also to obtain further information on the effects of different phenoxy acids. This new investigation gave an increase of the same magnitude in the risk for soft-tissue sarcomas after exposure to phenoxy acids or chlorophenols, but this risk related also to exposure to phenoxy acids free from impurities, such as polychlorinated dibenzodioxins and dibenzofurans. (Author abstract) (30 Refs)

37. THE DEVELOPMENT AND PROGNOSIS OF CHRONIC INTOXICATION BY TETRACHLORDIBENZO-P-DIOXIN IN MEN

Pardollera-Vejluphara J, Nemova M, Pichova J, Jirasek L, Lukas E

Faculty General Medicine, Charles Univ., Prague, Czechoslovakia

Arch Environ Health. 36(1):5-11 1981

During 1965 to 1968, 80 workers who had been engaged in the production of 2,4,5-sodium trichlorophenoxyacetate and butylester of trichlorophenoxyacetic acid became ill. The cause of the illness was 2,4,7,8-tetrachlorodibenzo-p-dioxin. A 10-yr study has been conducted for 55 exposed individuals. The majority of the patients developed chloracne, and 11 manifested porphyria cutanea tarda. Approx one-half of the patients suffered from metabolic disturbances, ie, pathologi-

cally elevated lipids with abnormalities in the lipoprotein spectrum, and two-fifths of the patients had pathological changes in the glucose tolerance test. One-third of the patients had biochemical deviations indicative of a mild liver lesion. Histological examination revealed light steatosis, or periportal fibrosis, or activation of Kupffer cells. Fluorescence of the liver tissues was present in UV light. In 17 persons symptoms of nervous system focal damage existed, with predominance of peripheral neuron lesion of the lower extremities (verified by EMG examination). The majority of patients suffered from various psychological disorders. As of this date, two patients have died of bronchogenic lung carcinoma; one of liver cirrhosis; one of a rapidly developed, extremely unusual type of atherosclerosis cerebri; and two patients have died in traffic accidents. The conditions of most other patients have improved. (Author abstract) (9 Refs)

38. THE HEALTH EFFECTS OF 'AGENT ORANGE' AND POLYCHLORINATED DIOXIN CONTAMINANTS

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Order Dept. OP 126, American Medical Assoc., P.O. Box 821, Monroeville, PA 15146

The Health Effects of 'Agent Orange' and Polychlorinated Dioxin Contaminants. Chicago, IL, American Medical Association, 30 pp., 1981

Medical evidence is presented regarding the toxicity and long-term effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a contaminant of Agent Orange, a 50:50 mixture of the n-butyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Agent Orange and TCDD are defined. Current medical and social concerns are addressed. Toxicological evidence of health hazards are presented based on animal (laboratory tests of toxicity, TCDD, picloram) and human (2,4-D, 2,4,5-T, silver, and TCDD) studies. Mutagenic and oncogenic effects of TCDD in humans, and its effects on reproduction and fertility in animals and humans are considered. Health surveys on the following populations are presented: Monsanto and Dow employees, Seveso inhabitants, BASF (Ludwigshafen, Germany) employees, NV Philips (The Netherlands) employees, Swedish railway workers, other Swedish workers, Finnish railway and forestry workers, Dow/Monsanto cohorts (soft-tissue sarcomas), other industrial workers, Vietnam war veterans, and 'dredging' personnel. The environmental fate of 2,4-D/2,4,5-T and TCDD is described. Critical review of the Environmental Protection Agency Ales studies are presented. Current and proposed studies are described. (190 Refs)

39. AGENT ORANGE TOXICITY: A QUANTITATIVE PERSPECTIVE

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Hum Toxicol. 1(1):31-39 1981

The toxic effect on Vietnam veterans from exposure to tetrachloro-dibenzo-p-dioxin (TCDD) from Agent Orange spraying operations was calculated using published data. Topics considered include the minimum toxic dose of TCDD in man, intake transfer factor, industrial cases and animal studies, and Vietnam. The dioxin sprayed with Agent Orange in Vietnam is thought insufficient to have caused systemic illnesses in Vietnam veterans or birth defects in their children. (35 Refs)

40. PHENOXY ACIDS, CHLOROPHENOLS, AND CANCER

Hendell L, Eriksson M
 Onkologiska kliniken, Region sjukhuset, Umeå, Sweden
 Lakartidningen. 79(54):2852-2863 1981

Recent studies on the carcinogenic effects of phenoxy acids and chlorophenols in occupationally exposed subjects are reviewed. Relationship between exposure to phenoxy acids and chlorophenols and malignant mesenchymal soft-tissue tumors was found in two case-control studies in Sweden. Increased risk of malignant lymphoma was also demonstrated in another study. Malignant lymphoma, acute leukemia, and malignant mesenchymal soft-tissue tumors were found at significantly increased incidence among workers exposed to (2,4,5-trichlorophenoxy)acetic acid 2,4,5-trichlorophenol, and the impurity 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD). TCDD was found to induce malignant mesenchymal soft-tissue tumors, and 2,4,6-trichlorophenol caused increased incidence of hepatocellular carcinoma, malignant lymphoma, and leukemia in rats. (15 Refs)

41. MORTALITY STUDY AND MORBIDITY SURVEY ON A COHORT OF WORKERS EXPOSED TO DIOXIN IN 1953 (MEETING ABSTRACT)

Frenzel-Beyers R
 German Cancer Research Center, Heidelberg, W. Germany
 Am J Epidemiol. 114(3):425 1981

A mortality study carried out on a cohort of 75 persons accidentally exposed to TCDD in 1953 at the BASF chemical plant, Ludwigshafen, revealed an increased risk of dying from stomach and lung cancer. A subsequent study was designed to evaluate the morbidity from existing records by a survey of information available from the occupational health system of the plant and other sources. For this purpose, the available medical records of the exposed cohort and of two matched comparison groups have been screened. Preliminary results showed increased findings of subjective and objective symptoms and disorders in the exposed group, but due to data protection problems it will not be possible to give a full appraisal of the findings until a later date. (2 Refs)

42. FIREFIGHTER EXPOSURE TO ORGANIC CARCINOGENS (MEETING ABSTRACT)

Bender S
 Bendix Environmental Research, Inc., San Francisco, CA
 J Occup Med. 23(4):305 1981

Burn, acute cardiac and respiratory hazards have been the past focus of attention to medical problems of firefighters. Firefighters are exposed to complex chemical mixtures of vaporized, off-gassed, decomposed and newly synthesized organic chemicals under conditions where they rarely know what they are breathing or what special chemicals hazards they may encounter. Self-contained breathing apparatus may not be worn, may not be available, or may not be properly maintained to preserve designed function. It is time to look at the long-term consequences of firefighter exposure to vinyl chloride, dioxins, urethane, benzene, tetrachloroethane, polyaromatic hydrocarbons and other carcinogens. Record-keeping and medical follow-up need improvement. (no Refs)

43. 2,4,5-T, TRICHLOROPHENOL, AND SOFT TISSUE SARCOMA (LETTER)

Hembar PA, Halperin WE
 Industry-wide Studies Branch, Div. Surveillance, Hazard Evaluations and Field Studies, Natl. Inst. Occupational Safety and Health, Cincinnati, OH. 45226
 Lancet. 1(8214):268-269 1981

In a review of four cohorts exposed to either 2,4,5-trichlorophenoxyacetic acid or to 2,4,5-trichlorophenol, 105 deaths were reported, three (2.9%) of which were due to soft tissue

sarcoma. This value is much higher than that expected for males in the United States, aged 20-64 yr. Taken alone, none of the cohorts showed an excess of risk for soft tissue sarcoma. When combined, however, the three cases suggest a common pattern. The combination of small cohorts of workers with a common occupational exposure for analyses is advocated. (6 Refs)

44. DON'T TAKE YOUR 'WORK' HOME WITH YOU

Bellin JS
 Control Action Div., Office Toxic Substances, United States Environmental Protection Agency, Washington, DC
 Occup Health Saf. 50(6):39-42 1981

The recognition that occupational exposure to toxic chemicals can affect the worker and his or her family is a new concept in work-related disease. Various routes of family exposure are illustrated with respect to worker exposure to anesthetic gases, lead, vinyl chloride, dioxin, polychlorinated biphenyls, methylmercury, diethylstilbestrol, ionizing radiation, asbestos, beryllium, kepone, and others. Data concerning beryllium and asbestos exposure are discussed in detail. (20 Refs)

45. THE MORTALITY EXPERIENCE OF WORKERS EXPOSED TO TETRACHLORODIBENZODIOXIN IN A TRICHLOROPHENOL PROCESS ACCIDENT

Zack JA, Siskind RR
 Dept. Medicine Environmental Health, Monsanto Co., 800 North Lindbergh Blvd., St. Louis, MO. 63166
 J Occup Med. 22(1):11-14 1980

A standardized mortality analysis was conducted on workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident at the Monsanto Company plant in Nitro, West Virginia. One hundred and twenty-one workers who developed chloracne resulting from this accident on March 8, 1969, were selected for study. Follow-up of this group was 100% complete. The standardized mortality ratio for all causes of death was shown to be 0.69, with 52 deaths observed and 46.41 expected. For the categories of malignant neoplasms and circulatory diseases, the standardized mortality ratios were 1.00 and 0.68, respectively. Because of the small size of the cohort and the relatively small number of deaths observed, the results of this study cannot be considered conclusive. However, it is important that no apparent excess in total mortality or in deaths from malignant neoplasms or diseases of the circulatory system was observed in a group of workers with a high peak exposure to tetrachlorodibenzodioxin, who were followed over a period of nearly 30 yr. The results of this study will be incorporated with those of a larger study which will include plant workers exposed to the course of 2,4,5-trichlorophenoxyacetic acid production during the period 1958 to 1969. (Author abstract) (13 Refs)

46. HERBICIDE EXPOSURE AND TUMOR MORTALITY. AN UPDATED EPIDEMIOLOGIC INVESTIGATION ON SWEDISH RAILROAD WORKERS

Axelsson O, Sundell L, Andersson K, Edling C, Hagstedt C, Kling H
 Unit Occupational Medicine, Univ. Hosp., S-581 85 Linköping, Sweden
 Scand J Work Environ. 6(1):73-79 1980

Follow-up of 348 Swedish railroad workers exposed to herbicides for more than 45 days during the period 1957-1972 has been extended through October 1978. The subjects were subdivided into cohorts with exposure to amitrol (3-amino-1,2,4-triazol), phenoxy acids, and combinations of the two. In 5,341 person-yr of observation, the total of all deaths was 45 observed vs 49 expected (the so-called healthy worker effect). Seventeen tumors were found vs the expected 11.85.

There was excess mortality from tumors, particularly noted among workers exposed to a combination of amitrol and phenoxyl acids (6 cases vs 1.78 expected). In the combined exposure group and the group exposed to phenoxyl acids alone, the total number of deaths was higher than expected, while in the amitrol group a lower number of deaths than expected was noted. The increase in deaths due to tumors occurred mainly among workers exposed in 1957-1961. An increase in overall tumor mortality was the trend among these workers, although no specific tumor type predominated (there were 3 stomach cancers and 3 lung cancers). It was suggested that these herbicides should be handled as increasingly suspect carcinogens. (14 Refs)

47. EPIDEMIOLOGICAL MORTALITY STUDIES IN THE CHEMICAL INDUSTRY BASF AKTIENGESellschaft, LUDWIGSHAFEN, FEDERAL REPUBLIC OF GERMANY (MEETING ABSTRACT)

Thoms AM, Loh R

BASF Aktiengesellschaft, Occupational Medicine and Health
Prevention, 6700 Ludwigshafen, Rhine, Federal Republic of
Germany

*English International Conference on Occupational Health in the
Chemical Industry, Sept 22-25, 1980, Tokyo Permanent
Commission and International Association on Occupational
Health, pp 56-60, 1980*

Epidemiological mortality studies have been undertaken in the BASF since 1974. The program covers prospective mortality studies on skilled chemical workers, emanating from exposure to health-impairing substances. A significant increase in the number of observed cases of cancer deaths in comparison to the expected cancer rate was only found in the trichlorophenol-dioxin cohort which was exposed to dioxin after an accident in 1953. A clear but not significant increase of observed against expected cancer deaths was found in the truck driver cohort. Only a slight increase in the number of cancer deaths could be observed in the cohorts with exposure to azurine, ortho-phthalodinitrile, vinyl chloride and vinylidene chloride. No increased rates of cancer deaths were found in the cohorts exposed to cadmium, chloroquinone, di-2-ethylhexylphthalate, ethylbenzene residue and styrene. Due to the high rate of mixed exposures it is difficult to evaluate the results in the case of acrylonitrile which is suspected to be a carcinogenic risk to man. (no Refs)

48. A MORTALITY ANALYSIS OF EMPLOYEES ENGAGED IN THE MANUFACTURE OF 2,4,5- TRICHLOROPHENOXYACETIC ACID

Ott MG, Halder BP, Olson RD

Medical Dept., Dow Chemical, Midland, MI, 48660
J Occup Med, 22(1):47-50, 1980

There have been few published reports regarding surveillance of individuals occupationally exposed to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The present study examined the mortality experience of 204 persons exposed to 2,4,5-T during its manufacture from 1950 to 1971. Length of employment in job assignments within the 2,4,5-T process area ranged from less than one yr to a max of approx 10 yr. Efforts to minimize 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) contamination of the product resulted in nondetectable concentrations using a method of detection developed in 1966 that was sensitive to 1 ppm. Within the scope of this mortality survey, no adverse effects were observed with respect to occupational exposure to 2,4,5-T or its feedstock, 2,4,5-trichlorophenol. (Author abstract) (9 Refs)

49. NEGATIVE CHEMICAL IONIZATION STUDIES OF HUMAN AND FOOD CHAIN CONTAMINATION WITH XENOBIOTIC CHEMICALS

Dougherty RC, Whistler MJ, Smith LM, Stalling DL, Kuehl DW
Dept. Chemistry, Florida State Univ., Tallahassee, FL, 32306
Environ Health Perspect, 36:103-117, 1980

The presence of toxic residues in fish and human tissue samples was studied through the use of negative chemical ionization mass spectroscopy (NCI-MS). The NCI-MS procedure routinely utilized either isobutane or methylene chloride (with traces of oxygen) as reagent gas mixtures. Clean-up of the fish and human tissue samples was achieved through steam distillation, gel-permeation chromatography, or gel-permeation chromatography followed by carbon-foam absorption chromatography (for planar aromatic hydrocarbons and their polychlorinated analogs). Analysis of samples of fish obtained from rivers and lakes in the United States revealed the presence of polychlorodibenzodioxins and polychlorodibenzofurans in many of the fish obtained at different locations. When the NCI-MS procedure was utilized for screening of human seminal fluid, initial results revealed the presence of many xenobiotics, including tetrachlorophenol, pentachlorophenol, polychlorinated biphenyls, and the dichlorodiphenyl-trichloroethane (DDT) metabolite DDMU. These results will ultimately be used in a study of potential correlations between low sperm densities and the occurrence of individual contaminants or groups of contaminants in seminal fluid. When the NCI-MS procedure was used for screening of human adipose tissues obtained at autopsy, preliminary results revealed the presence of hexachlorobenzene, polychlorinated biphenyls, heptachlor epoxide, nonachlor, chlordane, DDT, and the DDT metabolites DDMU and DDE; these results will ultimately be used in epidemiologic studies of environmental health problems. (31 Refs)

50. CONGENITAL HYPOTHALAMIC HAMARTOBLASTOMA, HYPOPHYSECTOMY, IMPERFORATE ANUS, AND POSTAXIAL POLYDACTYLY: A NEW SYNDROME? PART I: CLINICAL, CAUSAL, AND PATHOGENETIC CONSIDERATIONS

Hall JG, Palluaud JD, Clavens SK, Bachwitz JB, Wightworth FW,
Foster PC, Cho S, Bender PJ, Rind SD
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Center, 4800 Sand Point Way N.E., Seattle, WA
Am J Med Genet, 7(1):67-74, 1980

Six patients with what appears to be a new neonatally lethal malformation-dysplasia-IUGR syndrome are described. Symptoms of this syndrome include hypothalamic hamartoblastoma, postaxial polydactyly, imperforate anus, and other visceral anomalies. Hypopituitarism (5/6 patients) was characterized by microphallus, hypoadrenalism, and small thyroids. Three major types of anomalies with secondary, and perhaps tertiary effects were distinguished: 1) malformation-dysplasia complexes; 2) growth-cell division abnormalities; and 3) deformities. The hamartoblastomas (5/6 patients) contained neuronal elements in abnormal mixture and location, with neoplastic properties. It was concluded that the hamartoblastomas arose in the embryonic hypothalamic plate between days 34 and 40 or between the separation of the hypothalamic and thalamic plates and the formation of the supraoptic nucleus within the hypothalamic plate. The relationship of the tumors to the missing pituitary gland was uncertain. The patients were born over a 14 mo span in widely separated geographic areas of the continent. The pregnancy histories suggest excessive exposure to various drugs and toxic agents including Bendectin, Macrodonin, Norlestrin, Tylenol, Robitussin, Valium, phenobarbital, latex paint

fumes, the herbicide Agent Orange (2,4-D/2,4,5-T), fumes from a Styrofoam fire, B124 cleaning agent, and varnish remover. No two mothers shared the same exposure. The only common exposures were alcohol, cigarettes, and insecticide in three mothers. (39 Refs)

51. PATHOBIOCHEMICAL TRANSITION OF SECONDARY COPROPORPHYRINURIA TO CHRONIC HEPATIC PORPHYRIA IN HUMANS

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Klin Wochenschr 58(3):161-168 1980

Secondary coproporphyrinuria can be observed in one third of all patients affected by liver damage, especially by alcohol liver syndromes. Potentially, every secondary coproporphyrinuria can turn into a chronic hepatic porphyria, which progresses along several biochemically detectable latent phases and which indicates clinical manifestation by cutaneous symptoms. Two requirements are necessary for the transition of a secondary coproporphyrinuria to a chronic hepatic porphyria: 1) Genetic disposition of an autosomal dominantly inherited defect in uroporphyrinogen decarboxylase, which only becomes subclinically or clinically relevant through alcohol or estrogens (also oral contraceptives) in connection with liver damage; 2) A toxic inhibition of uroporphyrinogen decarboxylase in the liver caused by polyhalogenated hydrocarbons, such as hexachlorobenzene, polychlorinated and polybrominated biphenyls, dioxins and probably also methyl and vinyl chloride in combination with a present liver damage or caused by one of these agents. Experimental results, further, prove a direct negative effect of alcohol on the activity of uroporphyrinogen decarboxylase. In all liver conditions one should look out for porphyria, because it represents an important criterion for toxic liver damage and early diagnosis of a chronic hepatic porphyria is assessed by differentiating hepatic porphyrias. (Author abstract) (27 Refs)

52. DIOXIN DANGERS TO MAN

Hopfer J

No affiliation given
Food Cosmet Toxicol 18(5):541-545 1980

Patients with soft-tissue sarcomas were compared with a control group with respect to their exposures to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid, and chlorophenols. Four controls, matched for sex, age, and place of residence, were selected for each of 51 cancer patients. Exposure to these chemicals was associated with an almost sixfold increase in the risk of developing a soft-tissue sarcoma. When the phenoxyacetic acids were considered separately, the calculated increase in risk was slightly less. It was not possible from the study to separate the effects of the herbicides themselves from their dioxin or dibenzofuran impurities. Dioxin is one of the most toxic compounds known, and its content in 2,4,5-T typically used in the United States ranges from less than 0.01 ppm to 0.025 ppm. (14 Refs)

53. CLOUDS OF MISERY

Wright W

Monagh Hosp., Helston, Cornwall, England
Nurs Mirror 151(8):26-25 1980

The chemical dioxin (2,4,5 trichlorophenol), was used as a chemical agent during the Vietnam War and has been discharged accidentally into the environment by chemical plants manufacturing it for warfare and as a pesticide. The commonest reaction to dioxin is chloracne; some delayed reactions include cirrhosis of the liver, renal damage, and irreversible damage of a psychopathological/neurological

nature. In a South Vietnam hospital that serves the area heavily exposed to dioxin, the frequencies of stillbirths and the birth anomalies spina bifida and pure cleft palate were 2x and 3x higher than in areas not exposed to dioxin. Internal damage (including brain damage) can be caused by hexachlorophene, with which dioxin is linked. Long-term effects of dioxin have not yet been shown; however, thousands of animals were killed by dioxin or had to be destroyed after exposure. (no Refs)

54. 2,4,5-T AND CANCER (LETTER)

Kjallman T, Smith AH

Dept. Community Health, Univ. Auckland, Auckland, New Zealand
NZ Med J 91(658):318-319 1980

The relationship between 2,4,5-T (a chemical in Agent Orange) and cancer is discussed. Swedish research data indicate that action should be taken to prevent excessive human exposure to the chemical, and those involved with spraying should minimize the contact they have with the chemicals. Reports of cases of soft tissue sarcomas by practitioners in New Zealand to the cancer registry is suggested to carry out further epidemiological studies on 2,4,5-T. (12 Refs)

55. 245T - NOT ALL IN THE MIND (LETTER)

Ross S, Ross H

Open Univ., Walton Hall, Milton Keynes MK7 6AA, England
Trends Biochem Sci 5(9):15 1980

The author refutes the claim that the effects of agent orange (245T plus dioxin) are the result of hysteria produced by the media. A 1972 study of 98 refugees exposed to chemical sprays in South Vietnam is cited. The following effects were seen: effects on eyes and skin; gastrointestinal upset; fatigue; abortion; and human and animal teratogenicity. Deaths of exposed fish, fowl, and pigs were observed. (no Refs)

56. CASE-CONTROL STUDY: SOFT-TISSUE SARCOMAS AND EXPOSURE TO PHENOXYACETIC ACIDS OR CHLOROPHENOLS

Hardell L, Sundstrom A

Cancer Oncology, Univ. Hosp., S-901 85 Umea, Sweden
Br J Cancer 59(6):711-717 1979

Exposure to phenoxyacetic acids (PAA) and chlorophenols (CP) was studied in 52 patients with soft-tissue sarcomas (STS) and 208 controls. Thirty-one patients were deceased at the time of the study. Exposure to PAA or CP was registered in 36.5% of the STS patient and 9.2% of the controls. The relative risk for STS after exposure to PAA was calculated as 5.3. The relative risk associated with exposure to CP was 6.6. Four (7.7%) patients and 14 (6.8%) controls reported the use of paints containing CP during their leisure. Four patients and 14 controls also reported exposure to dichlorodiphenyltrichloroethane (DDT), the relative risk for which was calculated as 1.2. The risk of STS among unexposed persons involved in the same occupations as the exposed patients was 0.6, suggesting that the excess risk associated with PAA and CP was indeed related to these chemicals. There was no apparent difference in smoking habits between patients and controls. It is possible that the increased risk of STS associated with exposure to PAA or CP is caused by the pure chemical substances themselves and/or by impurities in the commercial preparations. (10 Refs)

57. THE SEVESO ACCIDENT: ITS NATURE, EXTENT AND CONSEQUENCES

Hamberger E, Reggiani G, Santib J, Woff HK

No affiliation given

Ann Occup Hyg. 22(4):527-570 1979

A runaway reaction during the commercial production of trichlorophenol in Seveso, Italy, resulted in the discharge of products containing 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD) over a 700-acre. The circumstances, causes, and extent of the accident are discussed. The effects of the accident on the health of the population included dermal and neurological findings, effects on organs and systems, the pregnant woman and the newborn child (abortions, congenital malformations, neonatal growth, and embryomorphological studies), cytogenetic and immunological studies, occurrence of infectious diseases, and birth rate and mortality. Considerations of effects on animals included the period immediately following the accident, conditions 2 months later (domestic animals and wildlife), precautionary slaughter, and analysis of tissue samples. Consideration of effects on crops and land included the situation after the accident, the behavior of TCDD in the environment, field and greenhouse trials, and decontamination methods. Pathology was related to the parameters of pregnancy, fetal development, growth of the newborn, immunoresponse, rate of chromosome aberrations, functions of the neurological system, hematological and hepatic conditions, morbidity, and the region. Chloracne, the skin lesion indicating TCDD exposure, appeared mainly in a small segment of the population, was generally mild, and was generally inclined to rapid and complete healing. (84 Refs)

58. APPROACHES TO THE EVALUATION OF GENETIC DAMAGE AFTER A MAJOR HAZARD IN CHEMICAL INDUSTRY: PRELIMINARY CYTOGENETIC FINDINGS IN TCDD-EXPOSED SUBJECTS AFTER THE SEVESO ACCIDENT.

Teschini ML, Crisomaldi C, Simonetti G, De Caroli L, Garipis R, Natta F

Istituto di Biologia Generale, Facoltà di Medicina, Università di Milano, Milan, Italy

Genetic Damage in Man Caused by Environmental Agents.

Proceedings of a Conference held in Oslo, Norway, May 11-13, 1977. Berg K, ed. New York: Academic Press, 551 pp., 1979.

Cytogenetic investigations were conducted on persons exposed to TCDD living in the Seveso area of Italy, the scene of a chemical accident. Data are presented on chromosome analyses of women exposed to the chemical who underwent therapeutic abortions in view of possible teratogenic effects. No significant change was noted in the chromosome numbers of any of the samples; however, marked fluctuations in the frequencies of chromosome lesions were noted in different subjects and different tissues. (7 Refs)

59. CASE CONTROL STUDY: MALIGNANT MESENCHYMAL SOFT-TISSUE TUMORS AND EXPOSURE TO PHENOXY ACIDS OR CHLOROPHENOLS (LETTER)

Hardell L, Sundström A

Umeå, Sweden

Laboratorium: 75(40):3535-3536 1978

Fifty-two men, aged 26-40 yr, with malignant mesenchymal soft-tissue tumors and 206 matched controls were surveyed by questionnaire for past occupational exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) and/or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The incidence of past exposure was 19/52 among the tumor patients vs 19/206 among the tumor-free controls. This difference was statistically significant (p less than 0.001). It was not possible to evaluate the risks of

2,4-D and 2,4,5-T separately, because most patients may also have been exposed to other chemicals, such as tetrachlorodibenzo-dioxin. These findings suggest that exposure to phenoxy acids, chlorophenols, and/or their impurities increases the risk of soft tissue tumors. (5 Refs)

60. CASE CONTROL STUDY OF MALIGNANT SOFT-TISSUE TUMORS AND EXPOSURE TO CHEMICAL SUBSTANCES (LETTER)

Eriksson JJ, Berg NO, Hardell L, Moller T, Axelson O

No affiliation given

Laboratorium: 76(44):3872-3875 1979

Levels of exposure to various chemical substances were determined in a retrospective case-control study of 110 men with malignant mesenchymal tumors and in 219 tumor-free men. (10 Refs)

61. PORPHYRIA CUTANEA TARDA

Mahlbauer JE, Pusthak MA

Dept. Dermatology, Harvard Medical Sch., Boston, MA

Int J Dermatol. 18(12):767-780 1979

The biochemistry, clinical presentation, differential diagnosis, laboratory findings, and histopathology of porphyria cutanea tarda (PCT) are reviewed. Environmental factors which may provoke PCT include alcohol, estrogens, iron, and chlorinated phenols. The treatment and photobiology of PCT and its relationship to hepatic tumors are discussed. (114 Refs)

II.

CARCINOGENICITY STUDIES ON DIOXINS AND DIBENZOFURANS IN EXPERIMENTAL ANIMALS

62. EFFECTS OF PCBs AND RELATED COMPOUNDS ON HEPATOCARCINOGENESIS IN RATS AND MICE

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Environ Health Perspect. 60:33-9 1983

Commercial mixtures of polychlorinated biphenyls (PCBs) and polychlorinated biphenyls (PBs) can cause hepatocellular carcinoma in rats and mice. Present evidence indicates that these chemicals act as promoters and not initiators of hepatocarcinogenesis. Our results show that Fireman BP-6 (FM) and its nontoxic major congener, 2,2', 4,4', 5,5'-hexabromobiphenyl (HBB), act as promoters in the two-stage model for hepatocarcinogenesis devised by Pitot and associates. A toxic congener, 3,3', 4,4', 5,5'-HBB, also was assessed for tumor-promoting activity. This congener, though not in FM, is similar to TCDD, in that both cause 3-methylcholanthrene (MC)-type induction of hepatic microsomal enzymes and produce similar toxic responses. FM contains several congeners which are mixed-type inducers in that they induce MC-type and phenobarbital (PB)-type enzymes. The toxicity of FM is most likely associated with its congeners which are mixed-type inducers and not to relatively nontoxic congeners such as 2,2', 4,4', 5,5'-HBB which are strictly PB-type inducers. Congener 3,3', 4,4', 5,5'-HBB acted as a tumor promoter only at a dose that was hepatotoxic. A synergistic effect on tumor promoting ability was produced by combining a nontoxic and nonpromoting dose of 3,3', 4,4', 5,5'-HBB with a promoting dose of 2,2', 4,4', 5,5'-HBB. Our results suggest that synergism between toxic and nontoxic congeners in FM may explain why mixtures such as FM have greater promoting ability than individual congeners. Our re-

sults also indicate that with PBB, toxicity and carcinogenicity are not necessarily related. (30 Refs)

63. PHENOXY AND PICOLINIC ACID HERBICIDES AND SMALL-INTESTINAL ADENOCARCINOMA IN SHEEP

Nowell KW, Riss AD, Renner RM

Clinical School of Medicine, Wellington, New Zealand

Lancet, 208(15):1501-5 1984

A study of age and sex matched groups of adult female sheep from 88 flat, hill, and high-country farms was conducted in the South Island of New Zealand to investigate the influence of breed and certain environmental factors on the prevalence rate of small-intestinal adenocarcinoma (SIA). 30 678 female sheep aged 5.5-7.5 years were examined at slaughter, 125 cases of SIA were found (6 per thousand) in animals from 61 farms (69%) and the prevalence rate for individual farm groups varied from 0 to 38 per thousand. Differences in tumour rate between breed groups were significant but differences between farm type were not. Exposure to phenoxy (Ph), picolinic acid (Pi) herbicides, or both (PhPi) was associated with significant increases in tumour rate. The increase in rate was significant for exposure to each of the 3 herbicide groups. Exposure to recently sprayed feed stuffs was associated with a significantly larger increase in tumour rate than exposure to less recently sprayed food. There was no difference between tumour rates of sheep exposed to Ph herbicides with or without 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Rates rose significantly with the total number of Ph, Pi, PhPi sprays used on the farm. The variation in rates associated with herbicides is sufficient to explain the breed differences recorded.

64. CARCINOGENICITY AND TOXICITY OF 2,4-DICHLOROPHENOXY-ACETIC ACID

Reuber MD

Sci Total Environ. 31(3):203-18 1983

2,4-Dichlorophenoxyacetic acid (2,4-D) is carcinogenic in male and female rats and probably also in mice. Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats. Male rats also had carcinomas of the endocrine organs. 2,4-D isopropyl ester was carcinogenic for the lymphoreticular system in female mice. 2,4-D and 2,4-dichlorophenol also were promoters of neoplasms of the skin in mice. Male mice given 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung. 2,4-D also is mutagenic and teratogenic in animals and causes poisoning in animals and human beings. (36 Refs)

65. TUMOR INITIATING ACTIVITY OF 9- AND 10-FLUORO-7,12-DIMETHYLBENZ[*a*]-ANTHRACENE (DMBA) AND THE EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON TUMOR INITIATION BY MONOFLUORO DERIVATIVES OF DMBA IN SENCAR MICE

DeGusman J, Derma PC, Diamond L

Wistar Institute of Anatomy and Biology, Philadelphia, PA

Carcinogenesis, 4(9):1045-9 1983

We have determined the skin tumor initiating activity in SENCAR mice of 6 monofluoro derivatives of 7,12-dimethylbenz[*a*]anthracene (DMBA). 9-Fluoro-DMBA (9-F-DMBA) was approximately as active, and 10-F-DMBA was more active than the parent hydrocarbon, DMBA. The difference between DMBA and 10-F-DMBA was most dramatic at the highest initiating doses of 10-F-DMBA tested. 4-F-DMBA, which was only weakly active as an initiator, was also tested as a complete carcinogen on mouse skin; after 50 weeks of treatment, 50- and 100-nmol weekly doses failed to

elicit papillomas or carcinomas. Animals treated with 50 nmol of DMBA weekly exhibited a 100% papilloma incidence and a 42% carcinoma incidence. Pretreatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) effectively inhibited tumor initiation with all of the monofluoro derivatives of DMBA tested. The ED₅₀ (dose of TCDD producing half-maximal inhibition) for the inhibition of DMBA initiation in SENCAR mice was determined to be 1.8×10^{-3} micrograms/mouse (5.6 pmol). The results indicate that the introduction of a fluorine atom in ring D of DMBA has no effect (positions 9 and 11) or enhances (position 10) tumor initiating activity. We believe 10-F-DMBA to be the first example of a hydrocarbon with a fluoro substituent giving rise to increased tumor initiating activity. The results also indicate that structural modifications that alter tumor initiating activity do not alter the ability of TCDD to inhibit tumorigenesis by DMBA.

66. ENHANCEMENT OF THE SKIN TUMOR-INITIATING ACTIVITY OF POLYCYCLIC AROMATIC HYDROCARBONS BY METHYL-SUBSTITUTION AT NON-BENZO 'BAY-REGION' POSITIONS

DeGusman J, Diamond L, Harvey RG, Siegel TJ

Wistar Institute of Anatomy and Biology, Philadelphia, PA

Carcinogenesis, 4(4):403-7 1983

The effect of substituting a methyl group at the non-benzo 'bay-region' site of several polycyclic aromatic hydrocarbons on skin tumor-initiating activity was determined. A methyl group at this position enhanced the tumor-initiating activity of dibenz[*a,h*]anthracene, 5-methylcholanthrene and 7-methylidibenz[*a,j*]anthracene, but not of dibenz[*a,c*]anthracene. 1,3,7,8-Tetrachlorodibenzo-p-dioxin was an effective inhibitor of skin tumor initiation by 7,14-dimethylidibenz[*a,h*]anthracene and 3,6-dimethylcholanthrene. There appears to be a general rule regarding methyl-substituted hydrocarbons: where a 'bay-region' exists in a polycyclic aromatic hydrocarbon molecule, methyl-substitution at the non-benzo 'bay-region' site results in enhanced tumor-initiating activity. The effect of methyl substitution in this position can be most simply explained as due to enhancement of the reactivity of the benzo ring through distortion of the aromatic ring system from planarity. The consequences of this effect are discussed.

67. CARCINOGENESIS BIOASSAY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (CAS NO. 1746-01-4) IN OSBORNE-MENDEL RATS AND B6C3F1 MICE (GAVAGE STUDY)

National Toxicology Program

Box 12233, Research Triangle Park, NC, 27709

Natl Toxicol Program Tech Rep Ser. Issue 200, 179 pp., 1982

Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin, (TCDD) a contaminant in several phenoxy herbicides, was investigated in groups of 50 male and female Osborne-Mendel rats and male B6C3F1 mice (0.01, 0.05, or 0.5 ug/kg/wk), and 50 female mice (0.04, 0.2, or 2.0 ug/kg/wk). TCDD was suspended in a vehicle of 9:1 corn oil-acetone and administered by gavage 2x/wk for 104 wk. A dose-related depression in mean body wt gain was observed in male and female rats, compared with groups of 75 vehicle-controls. Significant increases were observed in incidences of follicular-cell adenomas in the thyroid in male and female rats, neoplastic nodules of the liver in high-dose female rats, hepatocellular carcinomas in male and female mice, follicular adenomas in the thyroid in female mice, and incidences of toxic hepatitis related to administration of TCDD in high-dose rats and mice of each sex. Under the conditions of this

bioassay, TCDD was carcinogenic for both Osborne-Mendel rat and B6C3F₁ mice. (96 Refs)

68. CARCINOGENESIS BIOASSAY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (CAS NO. 1746-01-6) IN SWISS-WEBSTER MICE (DERMAL STUDY)

National Toxicology Program

Bldg 12233, Research Triangle Park, NC, 27709

Natl Toxicol Program Tech Rep Ser. Issue 261, 113 pp., 1982

Carcinogenicity of an acetone suspension of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) applied to the clipped backs of 30 male and female Swiss-Webster mice 3x/wk for 99 or 104 wk was investigated. Similar groups were treated with one application of 20 µg dimethylbenzanthracene (DMBA) in acetone 1 wk prior to TCDD administration. Females received 0.005 µg TCDD/application, and males 0.001 µg TCDD. Vehicle controls consisted of 45 mice of each sex treated with 0.1 ml acetone 3x/wk. Mean body wt of male dosed and vehicle control mice were less than those of untreated male controls throughout the study; for females, mean body weights were less than those of untreated controls for the first 80 wk. An increased incidence of pyelonephritis was observed in male mice exposed to acetone alone or in combination with TCDD. A significant increase in the incidence of fibrosarcoma of the skin was observed in female mice given TCDD and TCDD following DMBA compared with controls. Fibrosarcomas appeared significantly earlier in TCDD-dosed males than in vehicle controls, although the increased incidence of such tumors was not statistically significant. In male mice, the incidence of hemangiosarcoma was higher in the TCDD group than in the group given TCDD after DMBA, although no statistically significant differences in tumor incidences were noted between animals treated with TCDD alone and those treated with both TCDD and DMBA. Under the conditions used, TCDD was not carcinogenic for male Swiss-Webster mice, while it was for females, causing fibrosarcomas in the integumentary system. (96 Refs)

69. TUMOR PROMOTION BY TCDD IN SKIN OF HRS/J HAIRLESS MICE

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53706

Nature, 305(SUPP):271-273 1982

The capacity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to promote tumor formation in the skin of HRS/J hairless (hr/hr) mice and in their congenic, haired (hr/+) littermates was examined. Following initiation with dimethylbenz(a)anthracene, 12-O-tetradecanoylphorbol-13-acetate (TPA) promoted papilloma formation in the skin of both HRS/J haired and hairless mice, while TCDD was effective only in hr/hr mice. When HRS/J hairless mice were initiated with N-methyl-N'-nitro-N-nitrosoguanidine and treated twice weekly with various doses of TCDD or TPA, TCDD was found to be approx 100 times more potent than TPA on a molar basis. The capacity of several halogenated aromatic hydrocarbon congeners and commercial mixtures of halobiphenyl isomers to promote tumor formation in the skin of HRS/J mice was examined. The epidermal and systemic effects produced by repeated skin painting with TPA and TCDD were in sharp contrast. The incidence and multiplicity of tumor produced by initiation and TPA promotion were less in HRS/J hairless mice than in haired. Skin tumors elicited by promotion with TCDD were often smaller (1-3 mm) and progressed more slowly than those elicited by promotion with TPA. Usefulness of this model of tumor promotion in the skin of hairless mice is discussed. (37 Refs)

70. PROMOTING EFFECTS OF POLYCHLORINATED BIPHENYLS (AROCLO 1254) AND POLYCHLORINATED DEBENZOPURAN-FREE AROCLOR 1254 ON DIETHYLNITROSAMINE-INDUCED TUMORIGENESIS IN THE RAT

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Russell Ave., Madison, WI, 53706

JNCI, 66(3):309-315 1981

The hepatic tumor-promoting activity of a commercial polychlorinated biphenyl mixture, Aroclor 1254 (AR 1254), with and without its intrinsic polychlorinated dibenzofuran (PCDF) impurities, was investigated. Male Sprague-Dawley non-inbred albino rats were treated with 66 µg diethylnitrosamine (DENA)/ml drinking water for 5 wk and subsequently given a control diet or a diet supplemented (100 ppm for 18 wk) with either AR 1254 or AR 1254 from which the PCDF moieties were removed (AR 1254-PCDF). Of these animals receiving DENA alone, 16% exhibited hepatocellular carcinomas. Of those rats treated with DENA followed by administration of AR 1254 or AR 1254-PCDF, 64 or 84%, respectively, developed hepatocellular carcinomas. Tumor promotion with either AR 1254 or AR 1254-PCDF significantly (p less than 0.05) increased the incidence of DENA-initiated hepatocellular carcinomas. Administration of AR 1254 or AR 1254-PCDF alone did not induce hepatic tumors. Therefore, PCDF impurities were not necessary for the promoting activity of AR 1254. (Author abstract) (32 Refs)

71. BIOASSAY OF A MIXTURE OF 1,2,3,6,7,8- AND 1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXINS FOR POSSIBLE CARCINOGENICITY (GAVAGE STUDY)

Carcinogenesis Testing Program

Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, 20205

Natl Cancer Inst Carcinog Tech Rep Ser. (1982):1-173 1980

A mixture of 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (HCDD: 2 days/wk x 104 wk, gavage) was given to Osborne-Mendel rats and B6C3F₁ mice of both sexes. The HCDD dose was 2.5-10 µg/kg/wk in female mice and 1.25-5 µg/kg/wk in male mice and rats of both sexes. The rats showed a dose-related depression of body wt gain (after 68 wk of treatment in males, after 33 wk in females). The body wt gain in HCDD-treated mice was comparable to that of the vehicle (9:1 corn oil-acetone)-treated controls. No other toxic symptoms were observed, and HCDD had no adverse effect on survival in either species. Rats of both sexes developed hepatocellular carcinomas and neoplastic nodules; female rats also developed adenomas. Mice of both sexes developed hepatocellular carcinomas and adenomas. The incidence of liver tumors was dose-related in the HCDD-treated animals. Tumor incidence was significantly higher in HCDD treated female rats and in mice of both sexes, compared with the vehicle-treated controls, but not in male rats. HCDD also induced complex nonneoplastic toxic liver lesions in all groups of animals. Rats of both sexes also developed hyperplastic lung nodules after HCDD treatment. Under the conditions of this bioassay, HCDD was hepatocarcinogenic in female Osborne-Mendel rats and male and female B6C3F₁ mice; it was not demonstrated to be carcinogenic in male Osborne-Mendel rats. (27 Refs)

72. BIOASSAY OF 1,2,3,6,7,8- AND 1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXINS FOR POSSIBLE CARCINOGENICITY (DERMAL STUDY)

Carcinogenesis Testing Program

Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, 20205
Natl Cancer Inst Carcinog Tech Rep Ser. (1981):1-100 (1981)

Topical applications of a mixture of 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (HCDD: 0.005 ug, 5 days/wk x 16 wk, then 0.01 ug, 3 days/wk x 80 wk) were given to Swiss-Webster mice of both sexes. Some mice received one topical application of dimethylbenzanthracene (DMBA: 50 ug) 1 wk before the first application of HCDD. The untreated controls showed higher mean body wt than the HCDD-treated and vehicle (acetone)-treated mice; the body wt of the HCDD and vehicle groups was comparable throughout the 104-wk experiment. The frequency of alveolar/bronchiolar carcinomas of the lung was significantly higher in HCDD-treated male mice than in the vehicle-treated controls, but the difference was not significant between the HCDD-treated and untreated mice. The frequency of leukemias and lymphomas in HCDD-treated male mice was significantly lower than in the untreated controls. The frequency of fibrosarcomas of the skin was significantly higher in HCDD-treated female mice (with or without DMBA pretreatment) than in the untreated controls, but the difference was not significant between the HCDD-treated and vehicle-treated mice. Under the conditions of this bioassay, HCDD was not carcinogenic in Swiss-Webster mice of either sex. (26 Refs)

73. QUANTITATIVE EVALUATION OF THE PROMOTION BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN OF HEPATOCARCINOGENESIS FROM DIETHYLNITROSAMINE

Past HC, Goldworthy T, Campbell MA, Poland A

McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
Cancer Res. 40(10):3616-3620 (1980)

In order to test the potential of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a promoter of hepatocarcinogenesis, rats which had received a single 10-mg/kg dose of diethylnitrosamine (DEN) following partial hepatectomy were given TCDD (0.14 and 1.4 ug/kg sc once every 2 wk) for 7 mo. Animals which received (a) only a single initiating dose of DEN after partial hepatectomy and no further treatment or (b) TCDD alone with no initiating dose of DEN exhibited relatively few enzyme-altered foci and no hepatocellular carcinomas. However, animals initiated with DEN and then given TCDD had a marked increase in enzyme-altered foci. At the higher dose of TCDD, hepatocellular carcinomas were present in five of seven rats. By means of three different enzyme markers used to evaluate the phenotypes of the enzyme-altered foci, a distinct phenotype heterogeneity of the foci was noted with a shift towards phenotypes exhibiting a greater deviation from normal liver when TCDD was given following DEN-partial hepatectomy. Quantitation of the numbers of enzyme-altered foci was performed by relating measurements made from two-dimensional tissue sections to the numbers of foci per unit vol of liver using relationships established in the field of stereology. The total vol of the liver occupied by the enzyme-altered foci, but not their number, increased with the dose of TCDD administered following DEN-partial hepatectomy. These studies demonstrate that TCDD is a potent promoting agent for hepatocarcinogenesis. (Author abstract) (4) Refs

74. RESULTS OF A TWO-YEAR CHRONIC TOXICITY AND ONCOGENICITY STUDY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN RATS

Korsh RJ, Kryn DG, Beyer JE, Carver RM, Wade CE, Dittmer DA, Kunitz RP, Frouzan LE, Park CN, Barnard SD, Hummel RA, Hamilton CG

Toxicology Res. Lab., Health and Environmental Res., Dow Chemical Co., S.A., Midland, MI, 48660
Toxicol Appl Pharmacol. 66(2):279-305 (1978)

Groups of Sprague-Dawley rats were maintained for up to 2 yr on diets supplying 0.1 ug [2,193 parts per trillion (ppt)], 0.01 ug (208 ppt), or 0.001 ug (22 ppt) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)/kg/day. A dose of 0.1 ug/kg/day increased the incidence of hepatocellular carcinomas (female only) and squamous cell carcinomas of the lung, hard palate/nasal turbinates, and tongue, but it decreased the incidence of tumors of the pituitary, uterus, mammary gland, pancreas, and adrenal gland compared with controls. Morphological changes in hepatic, lymphoid, respiratory, and vascular tissue were seen by light and electron microscopy. The primary hepatic ultrastructural changes at this high dose level was proliferation of the rough endoplasmic reticulum. Other toxic effects included increased mortality, decreased wt gain, increased urinary excretion of porphyrins and delta-aminolevulinic acid, slight depression of erythroid parameters, and increased serum activities of alkaline phosphatase, gamma-glutamyl transferase, and glutamic-pyruvic transaminase. Rats treated with 0.01 ug/kg/day generally showed a lesser degree of toxicity, and treatment with the lowest dose caused no effects of toxicologic importance. Terminal liver and fat samples from rats of the three dosage groups contained 24,600 and 8,100, 5,100 and 1,700, and 540 and 540 ppt of TCDD, respectively. It is concluded that doses sufficient to induce severe toxicity increased the incidence of some types of neoplasms in rats and reduced the incidence of others. There was no increase in neoplasms in rats receiving sufficient TCDD during the 2-yr study to induce slight or no manifestations of toxicity. (9 Refs)

75. BIOASSAYS OF A MIXTURE OF 1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN AND 1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN (GAVAGE AND DERMAL STUDIES) FOR POSSIBLE CARCINOGENICITY: AVAILABILITY

Frederickson DS

Office Cancer Communications, NCI, NIH, Building 31, Room 16A21, Bethesda, MD, 20205
Fed Regist. 45(258):81126 (1980)

The administration of a mixture of 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin by gavage for 104 wk resulted in increased incidences of hepatocellular carcinomas or neoplastic nodules in female Osborne-Mendel rats and the induction of hepatocellular carcinomas and adenomas in male and female B6C3F1 mice. This mixture was not carcinogenic for male or female Swiss-Webster mice when applied under the conditions of this bioassay. (no Refs)

76. BIOASSAY OF 2,4,5-TRICHLOROPHOENOXYACETIC ACID FOR CARCINOGENICITY IN MICE

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Laboratoire de Genetique, Fondation Curie, Institut du Radium, 26, rue d'Ulm, Paris 75005
Br J Cancer. 33(6):625-633 (1976)

Adult C3Hf and XVII/G mice were given 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) by continuous po administration (80) ppm in the diet). The 2,4,5-T preparation contained less than 0.05 ppm of dioxins. Treated C3Hf mice showed a sig-

nificant increase in the incidence of neoplastic lesions. No significant difference was found in the XVII/G strain between the treated and control mice. Rare forms of tumors not observed in the controls were present in the 2,4,5-T-treated C3Hf mice including cutaneous tumors, a sebaceous squamous cell carcinoma, a squamous cell carcinoma, and an osteogenic tumor with a pulmonary metastasis. (18 refs)

III.

TOXICOLOGIC AND METABOLIC STUDIES ON DIOXINS AND DIBENZOFURANS IN EXPERIMENTAL ANIMALS

A.

TOXIC EFFECTS

77. TOXICITY AND FETOTOXICITY OF TCDD, TCDF AND PCB ISOMERS IN RHESUS MACAQUES (MACACA MULATTA)

McNulty WP

Environ Health Perspect. 60:77-88 1985

In rhesus macaques (*Macaca mulatta*), consumption of food containing commercial polychlorinated biphenyl (PCB) mixtures, some pure polychlorobiphenyl congeners, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) caused the same clinical toxic manifestations and histopathologic lesions, although the potencies of the toxicants covered a range of five orders of magnitude. Recovery from poisoning by 3,4,3',4'-tetrachlorobiphenyl (34TCB) or TCDF was rapid, whereas recovery from poisoning by Aroclor 1242, 3,4,3',4',5'-hexachlorobiphenyl (345HCB) or TCDD was protracted, if it occurred at all. 34TCB did not appreciably accumulate in body fat, but the level of 345HCB in fat rose steadily during ingestion. In one monkey, 25% of TCDD stored in fat after a single dose was still present after 2 years. Among the symmetrical tetra- and hexachlorobiphenyl isomers tested, subacute oral toxicity could be demonstrated only for those without ortho chlorine substitutions. 34TCB and 345 HCB were toxic at dietary levels of less than 1 ppm, but ingestion of food containing 2,5,2',5'-tetrachlorobiphenyl at 5 ppm, or 2,4,5,2',4',5'-, 2,4,6,2',4',6'-, or 2,3,6,2',3',6'-hexachlorobiphenyl at 15 or 65 ppm, caused no discernible deleterious effects. The principal demonstrable histopathological lesions, bone marrow excepted, were metaplasias in some specialized epithelial structures, such as sebaceous glands, nail beds, gastric mucosa, ameloblast, and thymic corpuscles. These changes were interpreted as toxicant-induced, reversible redirection of differentiation. This aberration was wholly reversible. TCDD and 34TCB caused abortions when given in one or a few oral doses early in pregnancy. At the total doses used (1 or 5 micrograms/kg of body weight for TCDD, 3 or 0.6 mg/kg of body weight for 34TCB), maternal toxicity was frequently apparent subsequent to the abortion.

78. MODULATION OF RAT HEPATIC MICROSOMAL TESTOSTERONE HYDROXYLASES BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND RELATED TOXIC ISOSTEROMERS

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Can J Physiol Pharmacol. 63:12:1537-42 1985

The effects of several toxic halogenated aryl hydrocarbons including 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran, 2,3,4,7,8-pentachlorodibenzofuran, 3,3',4,4'-tetrabromobiphenyl, 3,3',4,4'-pentachlorobiphenyl, and 3,3',4,4'-tetrachloroazobenzene on hepatic microsomal testosterone hydroxylases were determined in the immature male rat. All of these compounds induced hepatic testosterone 7 alpha-hydroxylase and inhibited testosterone 6 beta-, 16 alpha-, 16 beta-, and 2 alpha-hydroxylases and androstenedione formation. It was observed that there was a good correlation between the increase in testosterone 7 alpha-hydroxylase by the halogenated hydrocarbons and their ability to cause body weight loss in the exposed rats. Comparable linear correlations were observed between toxicity and the decreased activities of other testosterone hydroxylases. The role of altered testosterone metabolism in the toxicity of halogenated aryl hydrocarbons is unknown.

79. COMPARATIVE TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN FOUR (SUB)STRAINS OF ADULT MALE RATS

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Toxicol Appl Pharmacol. 77:314-30 1985

Four (sub)strains of adult male rats were given single oral doses of various concentrations of TCDD to establish and compare the oral 30-day LD50 values. The strains of rats were Fischer (F/334N) supplied by Harlan Industries, Frederick Cancer Research Center, and Charles River Breeding Laboratories; and CD supplied by Charles River Breeding Laboratories. The Charles River/Fischer rats were most sensitive to TCDD (LD50 = 164, 95% confidence limits 104-217 micrograms/kg), the Frederick/Fischer and Charles River/CD rats were moderately sensitive to TCDD (LD50 = 303, 250-360; and 297, 240-360 micrograms/kg, respectively), and the Harlan/Fischer rats were most resistant to TCDD (LD50 = 340, 281-409 micrograms/kg). The mean times of death were from 34.5 +/- 1.0 to 28.3 +/- 0.5 days and the percentage body weight loss at death was 37.4 +/- 1.2 to 42.7 +/- 1.3%. One week after exposure of the Charles River/Fischer animal to 45 micrograms TCDD/kg (1/4 the established 30-day LD50 dose), the same serum profile was induced as previously observed in the Harlan/Fischer rat, which includes hypoglycemia, hypertriglyceridemia, and hypercholesterolemia. These results emphasize the importance of indicating the precise dose, strain of rat, and time after dosing before termination in reporting the effects of TCDD on a particular biological response.

80. HEPATOTOXIC DOSES OF DIOXIN DO NOT DAMAGE MOUSE BONE MARROW CHROMOSOMES

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Mutat Res. 157:1:63-9 1985

We report on a study of the cytogenetic and hepatotoxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Mice of the C57B1/6J (with high-affinity TCDD receptor) or DBA/2J (with low-affinity TCDD receptor) strains were given single intraperitoneal injections of 50, 100 or 150 micrograms

of TCDD/kg body weight. At various times (8-48 h) after injection, we examined bone marrow cells for cytogenetic effects by performing structural aberration, sister-chromatid exchange, and micronucleus tests. 1 month after exposure, liver sections were studied for hepatotoxic effects. We found no evidence of chromosome damage by TCDD given in doses that cause liver damage in both strains of mice.

81. 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN-INDUCED CLEFT PALATE IN THE MOUSE: EVIDENCE FOR ALTERATIONS IN PALATAL SHELF FUSION

Probst R.M., Drouin L., Drouin V.M.
Teratogenesis: Carcinogen Mutagen; 9(5):427-436 1980

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) causes a high percentage of cleft palate in fetuses when administered during organogenesis in certain strains of mice including the C57BL/6J, but not in certain other strains (AKR/J). The purpose of the present study was to examine various biochemical and morphological aspects of TCDD-induced changes in the developing palatal shelves. Our results indicate that when TCDD (100 micrograms/kg) was given on individual days between days 8 and 10 of gestation, a high percentage of cleft palate was observed. Receptors specific for TCDD were detected in the C57BL/6J but not AKR/J palatal shelves. The amount of TCDD receptors is highest in the palatal shelves on day 13 as compared to other embryonic tissues including the liver. Examination of cryostat sections taken from embryos during the time of palatal elevation and fusion demonstrated that TCDD does not interfere with growth, elevation, or initial contact of the palatal shelves, but does interfere with firm adhesion and/or degeneration of the medial epithelial cells. Our results suggest that TCDD exerts a direct effect on the embryonic palatal shelves which results in formation of cleft palate.

82. IMMUNOLOGICAL TOXICITY OF CARCINOGENIC OR COCARCINOGENIC CHEMICALS

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Quo Vadis? Short-Term Tests for Carcinogens: Symposium held February 4-5, 1981, Montpellier, France, Clin. Mutat. Laboratories, p. 376-66, 1981.

Although within the concept of classical immune surveillance, immunosuppression by carcinogens has been considered to be an important contributory factor in carcinogenesis, it has been possible to dissociate the immunodepressive and carcinogenic activities by using carcinogen doses low enough to be devoid of immunosuppressive capacity or by using routes of administration that cause formation of tumor without systemic impairment of immunity. In a study of the effects of immunodepressive (eg, cyclophosphamide, doxorubicin, azathioprine, cyclosporin A) and immunotoxicologic (eg, 2,3,7,8-tetrachloro-dibenzo-p-dioxin, saccharin) xenobiotics on natural killer (NK) and macrophage cytotoxicities in experimental animals, and, to a lesser extent, in humans, it was found that rats exposed to 1-5% saccharin in their diet showed a dose-dependent impairment of humoral antibody production, but that, based on response to phytohemagglutinin, human lymphocytes were more resistant than rat lymphocytes to the in vitro immunosuppressive activity of the saccharin. The anatomical site under consideration, the level of xenobiotic at that site, the immune parameter examined, and the time of exposure to the xenobiotic during ontogenesis were found to be other determinants that critically affect the observed alterations of host defense mechanisms caused by the xenobiotics. Whatever the relevance of immunosuppression to carcinogenesis, the interaction with host defense mechanisms represents an important aspect of the toxicological properties of xenobiotics. (13 Refs)

83. EXAMINATION OF BONE MARROW, IMMUNOLOGIC PARAMETERS AND HOST SUSCEPTIBILITY FOLLOWING PRE- AND POSTNATAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

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Nat'l. Inst. Environmental Science, P. O. Box 12233, Research Triangle Park, NC, 27709
Int. J. Immunopharmacol. 2(4):301-310 1980

C57BL/6N pregnant mice (x C3H) were given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): 1.0, 5.0, or 15.0 ug/kg body wt) on day 14 of gestation and on days 1, 7, and 14 after birth and the effects of pre- and postnatal exposure on various immunologic parameters were studied. The pre- and postnatal 15.0 ug/kg dosing of mothers resulted in 70% fetal mortality, with the surviving offspring showing symptoms of overt toxicity. Bone marrow toxicity occurred in the offspring of mice receiving either 5 or 15 ug/kg TCDD, as shown by bone marrow hypocellularity and depressed colony formation of macrophage-granulocyte progenitor cells and pluripotent stem cells. The lymphoproliferative (LP) responses of offspring of mice receiving 5 and 15 ug/kg doses were depressed compared with control values following stimulation with T cell mitogens. LP responses to phytohemagglutinin and concanavalin A were suppressed 44% and 28% respectively, in T-cell enriched spleen cell fractions from TCDD-exposed offspring. Mice receiving either 1.0 or 5.0 ug/kg TCDD showed reduced survival of 40% and 70% respectively, compared with 28% for controls, after *Listeria monocytogenes* challenge, and an increase in tumor development to 60% and 44% respectively, compared to 22% in controls after challenge with PYB6 tumor cells. The depression of LP responses following mitogen stimulation in TCDD-immunosuppressed mice may be due to a functional defect in lymphocyte activation rather than in suppressor cell activity. (45 Refs)

84. CYTOGENETIC AND MORPHOGENETIC CHANGES IN OOGENESIS AND EMBRYOGENESIS INDUCED BY PYRIMETHAMINE AND 2,4,5-TRICHLOROPHENOXYACETIC ACID IN RATS IN PREOVULATORY MEIOTIC STAGE

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Res. Inst. Experimental Medicine, Acad. Medical Sciences, Leningrad, USSR
Genetika; 16(7):1220-1227 1980

Effects of pyrimethamine (chloridate) and 2,4,5-trichlorophenoxyacetic acid (administered to rats 9-12 hr before o-ulation) on the embryo mortality, cleavage rate, and frequency of chromosome aberrations were studied. It was concluded that the findings point to the mutagenicity of both compounds. (15 Refs)

85. PHENOXYACIDS AS INHIBITORS OF TESTICULAR DNA SYNTHESIS IN MALE MICE

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J. Environ. Contam. Toxicol. 21(1/2):99-92 1979

The abilities of five phenoxyacids to inhibit testicular DNA synthesis in male mice were studied. 2,4-Dichlorophenoxyacetic acid (2,4-D: 200 mg/kg, po), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T: 200 mg/kg, po), 2,4,5-T ester (200 or 400 mg/kg, po), 2-methyl-4-chlorophenoxyacetic acid (MCPA: 200 mg/kg, po), and 2-methyl-4-chlorophenoxypropionic acid (200 mg/kg, po) significantly depressed the uptake of labeled thymidine by testicular DNA. 2,4-Dichlorophenoxypropionic acid (200 mg/kg, po) did not significantly alter thymidine uptake, nor did 2,4,5-T ester at 50 or 100 mg/kg.

The mutagenic activity of three of the compounds was previously reported to decrease in the order MCPA greater than 2,4,5-T greater than 2,4-D; their ability to inhibit testicular thymidine uptake decreased in the same order. (15 Refs)

B.

INDUCTION OF MIXED FUNCTION OXIDASES

96. REGULATION OF RAT AND BOVINE ADRENAL METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS BY ADRENOCORTICOTROPIN AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Arch Biochem Biophys. 246(1):428-34 1986

The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on polycyclic aromatic hydrocarbon (PAH) metabolism and steroidogenesis in primary cultures of bovine adrenal cortical (BAC) and rat adrenal cortical (RAC) cells have been examined. Remarkably TCDD is an ineffective inducer (15-30%) of PAH metabolism in confluent BAC cells and completely antagonizes a 3-fold induction by benzo[a]anthracene (BA). In the same concentration range ($EC_{50} \approx 10^{-11}$ M) TCDD suppresses steroidogenesis through an effect on cholesterol metabolism. Adrenocorticotropin (ACTH) and cAMP also suppress PAH metabolism at concentrations which stimulate steroidogenesis (10^{-7} M). In RAC cells ACTH potently induces PAH metabolism (7-fold) at a comparable concentration to the stimulation of steroidogenesis. Parallel stimulation of PAH metabolism and steroidogenesis by cAMP suggest that ACTH induction of PAH metabolism is mediated by cAMP. TCDD induces PAH metabolism (2.8-fold, $EC_{50} \approx 10^{-11}$ M) at similar concentrations to the inhibitory effect in BAC cells and this action is additive with ACTH induction. In male rats in vivo TCDD induces adrenal microsomal PAH metabolism (72%) and is more effective in this respect than 3-methylcholanthrene (3MC). Rabbit antibodies against rat liver cytochrome P-450c (the major TCDD-inducible liver form) inhibited the TCDD-induced adrenal metabolism of 7,12-dimethylbenzo[a]anthracene (DMBA), which also exhibited regioselectivity typical of metabolism by P-450c. Constitutive adrenal microsomal metabolism, which exhibited regioselectivity of DMBA metabolism comparable to the ACTH-sensitive cellular metabolism, was not affected by anti-P-450c. It is concluded that ACTH and TCDD induce distinct forms of cytochrome P-450 in RAC cells and that the latter represents a typical Ah-receptor mediated response. The anomalous effect on PAH metabolism in BAC cells that parallels inhibition of steroidogenesis may derive from repression of a distinct adrenal form of P-450 by the TCDD-Ah-receptor complex.

97. REAPPRAISAL OF THE LIVER BENZOPYRENE HYDROXYLASE SYNTHESIZED DE NOVO AFTER TREATMENT OF RATS WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND 3-METHYLCHOLANTHRENE

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FEBS Lett. 198(2):425-8 1986

The dynamics of the inductive effects of MC and TCDD upon rat liver microsomal benzo[a]pyrene hydroxylase and the main properties of the de novo synthesized hemoproteins have been compared. The inadequacy of expression of the enzyme activity per total cytochrome P-448 content has been established. It was concluded that TCDD microsomes have a relatively low content of benzo[a]pyrene hydroxylase with a

higher molecular activity than the enzyme from MC microsomes.

98. THE RELATIVE INDUCTION OF MIXED-FUNCTION OXIDASE SPECIFIC ACTIVITY TO C-H AND C-C BOND STRENGTHS IN POLYCHLORINATED DERIVATIVES OF DIBENZO-P-DIOXIN (PCDDs)

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J Appl Toxicol. 6(1):67-71 1986

The structure-activity relationships between the mixed-function oxidase (MFO) system and six polychlorinated derivatives of dibenzo-p-dioxin (PCDDs) were studied using Sprague Dawley rats. The study was set up in accordance with previous work in these laboratories involving monocyclic hydrocarbons. Three possible mechanisms at the MFO active site are proposed. C-H bond hydroxylation in low chlorine substituted PCDDs, and probable epoxidation in the intermediate chlorinated species including 2,3,7,8-tetrachlorodibenzo-dioxin. Benzo[a]pyrene activity induced by some PCDDs appears irrelevant to the desired metabolic result.

99. GENETICALLY MEDIATED INDUCTION OF ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN MICE BY POLYCHLORINATED DIBENZOFURAN ISOMERS AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Arch Toxicol. 50(4):226-9 1983

Hepatic aryl hydrocarbon hydroxylase (AHH)-inducing potency of toxic polychlorinated aromatic hydrocarbons such as polychlorinated dibenzofurans (PCDFs), 3,4,5,3',4',5'-hexachlorobiphenyl (HCB) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was studied in four inbred strains of mice with different phenotypes of Ah locus, i.e., AHH-responsive strains: C57BL/6N and AKR/Ms QdJ, and AHH-nonresponsive strains: DBA/2Cr Slc and QdJ; DDD. Eight individual PCDF isomers or TCDD were administered IP in doses of 30 micrograms/kg; HCB was given in a dose of 120 micrograms/kg. In AHH-nonresponsive strains of mice, only TCDD significantly induced hepatic AHH activity, while in AHH-responsive strains, 2,3,7,8-tetrachlorodibenzo-furan(2,3,7,8-TCDF), 1,2,3,7,8-pentachlorodibenzo-furan(1,2,3,7,8-PCDF), 2,3,4,7,8-pentachlorodibenzo-furan(2,3,4,7,8-PCDF), and TCDD significantly enhanced the enzyme activity, and the induced AHH activities with the three PCDF isomers were about 30-65% of those of TCDD. These results indicate that AHH responsiveness in mice segregates with the induction of AHH activity by PCDF isomers and may also segregate with the toxic potency of the isomers; i.e., toxic potencies of 2,3,7,8-TCDF, 1,2,3,7,8-PCDF, and 2,3,4,7,8-PCDF in AHH-responsive strains of mice may be much greater than those in AHH-nonresponsive strains of mice. Taking into account both the potent AHH inducibility and the high bioaccumulation of 2,3,7,8-TCDF, 1,2,3,7,8-PCDF, and 2,3,4,7,8-PCDF, these three PCDF isomers should be given greater attention with regard to environmental contamination.

90. REGULATION OF AROMATIC OXIDATION OF ESTRADIOL-17 BETA IN MATERNAL HEPATIC, FETAL HEPATIC AND PLACENTAL TISSUES: COMPARATIVE EFFECTS OF A SERIES OF INDUCING AGENTS

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J Steroid Biochem; 22(4):563-7 1985

The effects of nine separate inducers of cytochrome P-450-dependent monooxygenases on the hydroxylation of estradiol-17 beta (E2) were investigated in near-term pregnant rats. Isoniazide exhibited highly effective inducing properties in the maternal liver (20-fold and 5-fold increases in 4- and 2-hydroxylase activities respectively). Pregnenolone 16 alpha-carbonitrile produced approx 20- and 30-fold increases in measured respective rates of 4- and 2-hydroxylase activities in fetal hepatic tissues; isoniazide produced only 2-fold increases in the same reaction. Only minor changes or slight increases in estrogen hydroxylation rates were observed in maternal hepatic, fetal hepatic or placental tissues following treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or other potent 3-methylcholanthrene (MC)-like inducing agents (beta-naphthoflavone, MC, caffeine). Phenobarbital exhibited relatively weak inducing properties and exposure of pregnant rats to ethanol from days 5-19 of gestation was without statistically significant effects on the parameters investigated. Rat placentas exhibited extremely low estrogen hydroxylase activities irrespective of pre-exposure of pregnant rats to the inducers studied. The results suggested separate regulatory controls for estrogen 2- and 4-monooxygenase activities even though relatively high correlation between the two reaction were generally observed in all three tissues.

91. INDUCTIVE EFFECT ON HEPATIC ENZYMES AND TOXICITY OF CONGENERS OF PCBs AND PCDFs

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Environ Health Perspect; 59:115-9 1985

The present paper describes a marked induction of liver microsomal cytochrome P-450 and cytosolic DT-diaphorase to cause possible disorder of steroid homeostasis and promotion of carcinogenicity of 4-nitroquinoline N-oxide (4-NQO) in rats by pretreatment with 3,4,5,3',4'-pentachlorobiphenyl (PenCB) or 2,3,4,7,8-pentachlorodibenzofuran (PenCDF). The animals were sacrificed 5 days after the pretreatment. These induction experiments showed that 7 alpha-hydroxylation of both progesterone and testosterone in liver microsomes was selectively increased to a great extent, but hydroxylation at the 2 alpha-, 6 beta- and 16 alpha-positions were depressed, together with 5 alpha-reduction. From the same microsomes, three of the strongly induced P-450 isozymes, i.e., high- and low-spin P-448s and P-452, were purified. The low isozyme was most responsible for 7 alpha-hydroxylation of testosterone. The pretreatment, also increased activity of DT-diaphorase and reduction of 4-NQO about 10-fold in liver 9000g supernatants. This reduction of 4-NQO was solely catalyzed by DT-diaphorase and the only product was 4-hydroxylaminoquinoline N-oxide, a proximate carcinogen, indicating that the pretreatment strongly increased production of a proximate carcinogen from 4-NQO. Such an enhancement of the metabolic activation of 4-NQO by the pretreatment was also observed to some extent in the lung and the skin. Persistence of PenCB and PenCDF in the liver of rats was also discussed.

92. INCREASES IN BENZO(A)PYRENE (BP) METABOLISM AND CYTOCHROME P-450(6) CONTENT IN ALVEOLAR TYPE II CELLS ISOLATED FROM TCDD-TREATED RABBITS (MEETING ABSTRACT)

Devereux TR, Dumas BA, Diliberto JJ, Phillips RM, Fouts JR

Natl. Inst. Environ. Hlth Sci., Rm. Triangle Park, NC 27709

Fed Proc; 44(4):1257 1983

Control rates of total BP metabolism in freshly isolated, sonicated alveolar type II cells from rabbits were 10-15 pmol/mg prot-1.min-1, about 1 pmol/10(6) cells-1.min-1. Treatment of rabbits with TCDD (10 ug/kg body wt in corn oil ip 4 days before killing) resulted in a 2- to 10-fold increase in the total BP metabolites formed by these cells. Although the amounts of all major metabolites were increased, the different metabolites were altered in varying degrees, the metabolites dependent on cytochrome P-450(6) increasing most. Cytochrome P-450(6) in microsomal preparations from the isolated cells, measured by protein blotting and immunostaining, was increased up to 40-fold by TCDD treatment of rabbits. 7-Ethoxycoumarin O-deethylase (7-ERFD) activity (dependent on this cytochrome P-450 isozyme) was increased similarly. Unexpectedly, the concentrations of cytochrome P-450(2) and NADPH cytochrome P-450 reductase activity were 2- to 3-fold higher in the microsomes of cells from TCDD-treated rabbits. Induction of cytochrome P-450(6) and 7-ERFD has been demonstrated in pulmonary microsomes from TCDD-treated rabbits, although increases in BP metabolism, cytochrome P-450(2) and cytochrome P-450 reductase have not been observed in these preparations. Whether or not all of the increases seen in the type II cells are caused by an inductive effect of TCDD remains to be elucidated.

93. EFFECTS OF CHLORINATED DIBENZOFURAN AND DIOXIN ON CONCENTRATION AND PATTERN OF CHLOROBIPHENYLS AND ACTIVITY OF BENZO(A)PYRENE HYDROXYLATION IN MICE

Nagayama J, Kiyohara C, Honda S, Matsuda Y, Kawasumi M

Fukuoka Igaku Zasshi; 76(3):173-83 1983

Keywords (MeSH): Adipose Tissue/metabolism; Animal; Aryl Hydrocarbon Hydroxylases/*metabolism; Benzo(a)pyrene/*pharmacodynamics; Dioxins/*pharmacodynamics; Liver/enzymology/metabolism; Lung/metabolism; Male; Mice; Mice, Inbred Strains; Polychlorobiphenyl Compounds/*metabolism.

94. PARTICIPATION OF CYTOCHROME P-448 IN 3,4-BENZOPYRENE METABOLISM DURING INDUCTION BY DIFFERENT XENOBIOTICS IN INBRED MOUSE STRAINS

Mishina DV, Mishin VM, Lashchinskii VV

Dokl Akad Nauk SSSR; 283(2):487-9 1985

Keywords (MeSH): Animal; Antibodies/immunology; Benzo(a)pyrene/*metabolism/pharmacodynamics; Comparative Study; Cytochromes/*biosynthesis/immunology; Enzyme Induction/drug effects; Immunization; Male; Methylcholanthrene/pharmacodynamics; Mice; Mice, Inbred AKR/*metabolism; Mice, Inbred C57BL/*metabolism; Mitochondria, Liver/drug effects/enzymology/immunology; Tetrachlorodibenzodioxin/pharmacodynamics.

95. CORRELATION BETWEEN CYTOCHROME P-448 CONTENT AND BENZOPYRENE HYDROXYLASE ACTIVITY DURING THE INDUCTION OF MICROSOMAL MONOOXYGENASES USING METHYLCHOLANTHRENE-TYPE XENOBIOTICS

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Biochemica, 49(3):464-9 1984

Using antibodies against electrophoretically homogeneous cytochrome P-448 from rat liver microsomes induced by 3-methylcholanthrene, the changes in the immunologic identity and contents by cytochrome P-448 induced by 3-methylcholanthrene, 3,4-benzopyrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), were studied. No cytochrome P-448 was detected in the liver microsomes of control or phenobarbital-induced rats. This form of the cytochrome makes up to about 35% of the total content of the CO-binding hemoprotein during TCDD induction and up to 90% during 3-methylcholanthrene and 3,4-benzopyrene induction. On the other hand, 3-methylcholanthrene, 3,4-benzopyrene and TCDD significantly and equally activates the cytochrome P-448-dependent benzopyrene hydroxylase, since the antibodies against cytochrome P-448 inhibit benzopyrene metabolism in the microsomes by 85-90%. The possible reasons for the TCDD-induced increase in the catalytic activity of cytochrome P-448 as compared to the immunologically identical cytochrome P-448 induced by 3-methylcholanthrene and 3,4-benzopyrene, are discussed.

96. METABOLISM OF BENZO(A)PYRENE BY CYTOCHROME P-450, FORM 6, IN RABBIT LUNG (MEETING ABSTRACT)

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Fed Proc., 43(3):544 1984

Solubilized microsomes from lungs of untreated and TCDD-treated rabbits (where cytochrome P-450, form 6, is approximately 3% and 10-20% of the total, respectively) were chromatographed on DEAE cellulose using a 0 to 0.25 M KCl gradient. Column eluates were assayed for form 6 by 7-ethoxycoumarin-O-deethylation activity in a reconstituted monooxygenase system and by immunochemical detection. With control microsomes, form 6 eluted in the trailing edge of the form 5 fraction. Immunochemical analysis showed that 97% of the total form 6 content was in the trailing edge. In the chromatography of microsomes from lungs of TCDD-treated rabbits, form 6 eluted as a distinct fraction following the form 5 fraction. Analysis of four combined fractions encompassing the two eluted isotypes showed less than 10% cross-contamination. Purified cytochrome form 6 metabolized benzo(a)pyrene in a reconstituted rabbit monooxygenase system and this activity was inhibited by alpha-naphthoflavone. The turnover number for form 6 catalyzed metabolism of benzo(a)pyrene was approximately 4-fold greater than that for form 2. Preparations of form 5 uncontaminated with form 6 (as evidenced by immunochemical detection of Western blots) did not metabolize benzo(a)pyrene in this system. These findings indicate that the results of all earlier studies on the activity of form 5 with benzo(a)pyrene were due to the presence of small amounts of cytochrome P-450, form 6.

97. INDUCTION OF CYTOCHROME P-450 DEPENDENT REACTIONS IN THE RAT VENTRAL PROSTATE BY BETA-NAPHTHOFLAVONE AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Toxicology, 29(1-2):61-75 1983

The purpose of this study was to characterize drug metabolism in the rat ventral prostate in untreated, beta-naphthoflavone (BNF) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) treated animals. Intraperitoneally administered [³H]benzo(a)pyrene (BP) was recovered in about equal quantities in rat ventral prostate, lungs and testes. The relatively high uptake of [³H]BP in the ventral prostate might possibly be related to the occurrence of a macromolecule in the gland binding TCDD and 3-methylcholanthrene (MC) with a relatively high affinity (K_d 1.5 and 5.0 micromol, respectively). BNF, BP and MC competed with the TCDD binding site of the macromolecule. The prostatic cytochrome P-450 dependent microsomal enzyme activities aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin O-deethylase are very low in untreated animals (about 1 pmol/min/mg of microsomal protein). BNF- or TCDD treatment of the animals induced these activities about 500-fold, whereas aminopyrine N-demethylase was enhanced to a smaller degree. Maximal enzyme activities of AHH and 7-ethoxycoumarin O-deethylase were obtained 24 h after a single intraperitoneal injection of BNF and the activities decayed relatively rapidly after this peak. The magnitude of enzyme induction was much higher in the prostate than in the liver. Mutagenic BP metabolites were formed *in vitro* by induced prostatic microsomes as revealed by HPLC analysis. The activity of NADPH-cytochrome c reductase was 35 pmol/min/mg and was not changed by BNF- or TCDD treatment.

98. IMMUNOLOGICAL AND ENZYMATIC COMPARISON OF HEPATIC CYTOCHROME P-450 FRACTIONS FROM PHENOBARBITAL-, 3-METHYLCHOLANTHRENE-, BETA-NAPHTHOFLAVONE- AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN-TREATED RATS

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Necker-Enfants Malades, Paris, France.
Biochem Pharmacol., 31(1):1673-82 1983

A comparison of the cytochrome P-450 forms induced in rat liver microsomes by phenobarbital on the one hand, and 3-methylcholanthrene, beta-naphthoflavone and 2,3,7,8-tetrachlorodibenzo-p-dioxin on the other hand, was performed using specific antibodies: anti-P-450 B2 PB IG (against the phenobarbital-induced cytochrome P-450) and anti-P-450 B2 BNF IG (against the beta-naphthoflavone-induced cytochrome P-450). On DEAE-cellulose chromatography, four cytochrome P-450 fractions were separated, called P-450 A (non-adsorbed), P-450 Ba, P-450 Bb and P-450 Bc, from control, phenobarbital-, 3-methylcholanthrene, beta-naphthoflavone- and 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats. Cytochrome P-450 A fractions appeared to be unmodified by the inducers, whereas the specifically induced cytochrome P-450 forms were always recovered in Bb fractions. The P-450 Bb fractions induced by 3-methylcholanthrene, beta-naphthoflavone and 2,3,7,8-tetrachlorodibenzo-p-dioxin exhibited common antigenic determinants, comparable catalytic activities (benzphetamine N-demethylase, benzo(a)pyrene hydroxylase) and similar mol. wts. Moreover, the inhibition patterns by the two antibodies of benzphetamine N-demethylase and benzo(a)pyrene hydroxylase activities catalysed by 3-methylcholanthrene, beta-naphthoflavone and 2,3,7,8-tetrachlorodi-

benzo-p-dioxin microsomes or by the corresponding P-450 Bb fractions in a reconstituted system were quite identical. By these different criteria, benzo-naphthoflavone, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin seem to induce a common cytochrome P-450 species in rat liver.

99. EFFECT OF POLYCYCLIC AROMATIC COMPOUNDS AND PHORBOL ESTERS ON ORNITHINE DECARBOXYLASE AND ARL HYDRO CARBON HYDROXYLASE ACTIVITIES IN MOUSE LIVER

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Single ip injections of 3-methylcholanthrene (MC; 50 mg/kg) administered to inbred C57BL/6 mice or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 100 µg/kg) to DBA/2 mice gave an increase in the hepatic activities of ornithine decarboxylase (ODC) and aryl hydrocarbon hydroxylase (AHH) with peaks occurring by 12 and 48 hr, respectively. A single ip dose of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA; 100 µg/kg) enhanced the activity of ODC about 70-fold within 12 hr in C57BL/6 mice and 18-fold within 24 hr in DBA/2 mice without affecting AHH activity markedly. 4-O-Methyl-12-O-tetradecanoylphorbol-13-acetate (100 µg/kg) raised ODC activity to about 25% of the TPA-treated value in C57BL/6 mice; in DBA/2 mice, TPA and 4-O-methyl-12-O-tetradecanoylphorbol-13-acetate induced ODC activity to roughly the same level. Benzo(e)pyrene (50 mg/kg) failed to affect ODC and AHH activities significantly in either strain. The inducing effect of TPA on ODC activity was potentiated by a simultaneous administration of MC to C57BL/6 mice; combined TPA and TCDD to DBA/2 mice exerted an additive effect on hepatic ODC activity. Difluoromethylornithine administered ip effectively inhibited the induction of ODC activity elicited by TPA, MC, or TCDD either alone or in various combinations but did not interfere with AHH induction. These data indicate that different regulatory factors are involved in the ODC induction process elicited by TPA and polycyclic aromatic compounds and that MC and TCDD may induce ODC activity by different mechanisms. The results also confirm our earlier findings in rat skin and cells in culture which suggest that the ODC and AHH induction processes can occur independently of each other. Additionally, there is a strain-related difference in sensitivity with regard to ODC-inducing activity of TPA in the livers of C57BL/6 and DBA/2 mice. (Author abstract) (41 Refs)

100. KINETICS OF N- AND C-HYDROXYLATIONS OF 2-ACETYLAMINOFLUORENE IN MALE SPRAGUE-DAWLEY RAT LIVER MICROSOMES: IMPLICATIONS FOR CARCINOGENESIS

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Cancer Res. 43(8):3720-4 (1983)

The metabolism of 2-acetylaminofluorene (AAF) has been studied in male Sprague-Dawley rat liver microsomes over a concentration range of 0.02 to 300 µM, and kinetic parameters have been determined for five oxidative pathways. The N-hydroxylation of AAF was best described by a single enzyme system with a mean K_m of 0.033 µM and a mean V_{max} of 3.63 pmol/mg/min. Pretreatment of animals with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) caused a marked induction of N-hydroxylase activity while phenobarbital had no effect. Biphasic kinetics for the 7-hydroxylation of AAF were observed in both control and TCDD- and phenobarbi-

tal-induced microsomes. The high-affinity K_m [0.031 $\pm$ 0.015 (S.E.) µM; $n = 3$] in control microsomes was 3 orders of magnitude lower than the low-affinity K_m [103 $\pm$ 16 µM; $n = 3$] indicating that each isoenzyme predominated at vastly different substrate concentrations. The mean V_{max} values for the low- and high-affinity enzymes were 3.5 and 1351 pmol/mg/min, respectively. TCDD pretreatment markedly induced the activity of the low-capacity enzyme and reduced the activity of the high-capacity enzyme. Phenobarbital caused a significant induction of both enzyme pathways. Biphasic kinetics were also observed for the 5-, 3-, and 1-hydroxylations of AAF in control and phenobarbital-induced microsomes, but in TCDD-pretreated microsomes only 1-hydroxylation exhibited biphasic kinetics. TCDD caused a marked induction of these metabolic pathways while phenobarbital had no effect. Nonclassical kinetics were observed for the 9-hydroxylation of AAF, and at high substrate concentrations detoxification via this pathway and 7-hydroxylation predominated. However, at low concentrations, metabolic activation of AAF via N-hydroxylation was a major pathway. These data indicate that multiple forms of cytochrome P-450 are involved in AAF metabolism and that the balance between metabolic activation and detoxification of this substrate is dependent on both concentration and previous exposure to inducers.

101. SOME BIOTRANSFORMATION ENZYMES RESPONSIBLE FOR POLYCYCLIC AROMATIC HYDROCARBON METABOLISM IN RAT NASAL TURBINATES: EFFECTS ON ENZYME ACTIVITIES OF IN VITRO MODIFIERS AND INTRAPERITONEAL AND INHALATION EXPOSURE OF RATS TO INDUCING AGENTS

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Cancer Res. 43(10):4005-11 (1983)

Respiratory tract biotransformation of many xenobiotics found in inhaled environmental pollutants is generally considered essential for the mutagenic, carcinogenic, and/or toxic response of lung tissue to these xenobiotics. Typical environmental pollutants contain known carcinogens adsorbed onto particles which can deposit in the nasal pharyngeal region of the respiratory tract. The purpose of this study was to characterize the metabolic capacity of rat nasal tissue. Both oxidative and nonoxidative enzyme activities were investigated which included aryl hydrocarbon hydroxylase (AHH), epoxide hydrolase (EH), uridine 5'-diphosphate-glucuronyltransferase (UDPGT), and glutathione transferase. Specific enzyme activities of AHH, EH, UDPGT, and glutathione transferase were 0.023, 6.4, 20.4, and 24.8 nmol product per mg protein per min, respectively. Benzo(a)pyrene was metabolized by AHH to dihydrodiols, quinones, and phenols in quantities which were about 10 times greater than those reported for rat lung microsomes. Small, but detectable, quantities of benzo(a)pyrene tetrols were also measured in reaction flasks in which rat nasal tissue was incubated with benzo(a)pyrene. Attempts to increase the microsomal enzyme activities of AHH, EH, and UDPGT by pretreating rats with various inducing agents by both i.p. injection (phenobarbital, 3-methylcholanthrene, Aroclor 1254, and 2,3,7,8-tetrachlorodibenzo-p-dioxin) and inhalation exposure (BaP) resulted in rat nasal monooxygenases only being induced (2-fold) after pretreatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Phenobarbital increased enzyme activities of EH and UDPGT by about 50%. These data suggest that rat nasal tissue may contain multiple forms of cytochrome P-450 and of EH and UDPGT. The results from this study support the

notion that nasal tissue may be important in determining the metabolic fate of inhaled xenobiotics.

182. DEVELOPMENT OF BASAL AND INDUCED ARYL HYDROCARBON (BENZO[*a*]PYRENE) HYDROXYLASE ACTIVITY IN THE CHICKEN EMBRYO IN OVO

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The development of the hepatic microsomal mixed-function oxidase system was studied to determine the basal level of embryonic enzyme activity and the inducibility of this system throughout growth and differentiation. Chicken embryo livers were assayed for basal and inducible hepatic aryl hydrocarbon hydroxylase (AHHase; designated elsewhere as AHH) activity from the first appearance of the liver as a discrete organ at 5 days of incubation (DI) through day 10 after hatching. In addition, whole-embryo and viscera preparations were assayed at 3 and 4 DI. Basal AHHase activity was equal to or greater than adult levels from 3 DI through hatching in all preparations (approximately 0.3-0.5 nmol/min per mg). A 3-fold increase in basal activity above adult values occurred at hatching. The onset of inducibility in chicken embryo liver between 5 and 6 DI was concomitant with hepatocyte differentiation. A developmental profile of 24-hr 3,4,3',4'-tetrachlorobiphenyl-induced AHHase activity showed 15- to 30-fold induction over controls from 7 DI through day 10 after hatching, with a maximum of 15 nmol/min per mg at 14 DI and day 1 after hatching, a specific activity greater than 50% greater than maximal induction in the adult. Embryonic AHHase activity was also induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin, 3-methylcholanthrene, beta-naphthoflavone, and sodium phenobarbital. Induction kinetics throughout embryonic development were similar to those reported for the adult chicken and other animals. These findings demonstrate development of a mixed-function oxidase system in very early embryogenesis and then in the liver as it differentiates. Liver AHHase activity is inducible throughout development and perinatally but such activity is under strict developmental regulation. The chicken embryo has adult levels of AHHase activity which would be sufficient to achieve metabolic activation of promutagens/carcinogens before and after hepatocyte differentiation.

185. COMPARISON OF THE INDUCIBILITIES OF UDP-GLUCURONOSYLTRANSFERASE AND POLYSUBSTRATE MONOOXYGENASE ACTIVITIES IN MICE EXPOSED TO 20 MODEL XENOBIOTICS

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Keywords (MeSH): Animal; Aryl Hydrocarbon Hydroxylase/*genetics; Enzyme Induction; Kinetics; Male; Methylcholanthrene/pharmacodynamics; Mice; Mice, Inbred C57BL; Microsomes, Liver/drug effects/*enzymology; Phenobarbital/pharmacodynamics; Polychlorobiphenyl Compounds/pharmacodynamics; Tetrachlorodibenzo-dioxin/pharmacodynamics; UDP Glucuronosyltransferase/*genetics.

186. HEPATIC ACCUMULATION OF PCDF AND ITS ACTION ON ENZYME INDUCTION IN PCB-INTOXICATED MONKEYS

Kuroki H, Maeda Y, Kaga N, Yoshimura H

Pohanka Igohu Zaitch; 74(5):234-9 1981

Keywords (MeSH): Animal; Benzo-furans/*metabolism; Benzo-pyrene Hydroxylase/biosynthesis; English; Abstract; Enzyme Induction/drug effects; Liver/drug effects/*metabo-

lism; Macaca; Polychlorobiphenyl Compounds/*poisoning; Quinone Reductases/biosynthesis.

189. CHEMICAL ANALYSIS AND TOXICITY OF POLYCHLORINATED BIPHENYLS AND DIBENZOFURANS IN RELATION TO YUSHO

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J Toxicol Sci; 7(3):161-75 1982

A typical lot of Kanemi rice oil ingested by patients with Yusho (PCB poisoning) and the blood, liver and adipose tissue of the patients were analyzed for individual congeners of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) by gas chromatography and gas chromatography-mass spectrometry. The individual congeners identified were assayed for biological properties such as accumulation ability in the liver of monkeys and rats, inducing activities of benzo[*a*]pyrene 3-hydroxylase, benzo[*a*]pyrene demethylase and DT diaphorase in rats, and gravimetric changes of the thymus and liver in rats. Among the seven PCB congeners detected in Yusho patients, 2,3,4,5,3',4'-hexa-CB seems to be the most related compound to Yusho by its strong effects on induction of the liver enzymes, and on atrophy of the thymus and hypertrophy of the liver in rats. PCDF congeners identified in the patients showed severe toxicity in rats than the PCB, exhibiting stronger enzyme induction and gravimetric changes of the tissues even at very low doses of 1-10 micrograms/kg. These PCDFs, especially 2,3,4,7,8-penta-CDF, were also very accumulative in the liver. Therefore, they are considered as the most important etiologic agents for current symptoms of Yusho. (33 Refs)

190. STUDIES ON THE EFFECT OF INTRAGASTRICALLY ADMINISTERED 3-METHYLCHOLANTHRENE ON INTESTINAL MIXED-FUNCTION OXIDASE ACTIVITY AND PROTEIN SYNTHESIS

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Dis Assoc Int JBL; 45(2):408-8 1982

Various polycyclic aromatic hydrocarbons including 3-methylcholanthrene (MC), benzo[*a*]anthracene (BA), Aroclor 1254 (ARO) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), were compared for their efficacy of induction of intestinal microsomal aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin O-deethylase and cytochrome P-450, in rats 18 h following one intragastric dose. TCDD treatment produced the greatest induction of the specific MFO (mixed function oxidase) activities tested, followed closely by MC treatment. Rank order of efficacy of induction of spectrally determined cytochrome P-450 was TCDD, ARO, MC and BA. Time-course studies demonstrated an MC-mediated effect on AHH activity as early as 3 h which peaked at 18 h. The sensitivity of the MC-mediated induction of AHH activity to pretreatment with inhibitors of protein and RNA synthesis was studied. Cordycepin significantly inhibited the induction at 12 h but not at 3 h post-treatment with MC. Both cycloheximide and actinomycin D significantly inhibited the MC-mediated induction at 3 and 12 h after MC treatment. An MC-mediated stimulation of 3H-leucine incorporation into microsomal protein in vivo was observed at 12 h following MC treatment. A cell-free protein synthesis employing intestinal ribosomes was developed. The rate of protein synthesis (3H-leucine incorporation/ug rRNA/3 min) and the number of active ribosomes (3H-peptidyl-puromycin formed/ug rRNA) were determined. No effect of in vivo MC treatment was observed in subsequently isolated intestinal ribosomes at either 3, 9 or 18 h post-treatment. Attempts to localize an effect of MC

pretreatment in ribosomes isolated from the upper villous or lower crypt regions of the mucosal epithelium or upon free vs membrane-bound ribosomes was unsuccessful. (no Refs)

107. METABOLISM OF THE PANCREATIC CARCINOGEN N-NITROSO-2,6-DIMETHYLMORPHOLINE BY HAMSTER LIVER AND COMPONENT CELLS OF PANCREAS

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Cancer Res. 42(12):5089-5095 1982

The compound N-nitroso-2,6-dimethylmorpholine (NNDM) is an indirect nitrosamine carcinogen which induces ductal adenocarcinoma of the pancreas in the Syrian golden hamster. NNDM is oxidized to give three major metabolites. The major products are N-nitrosobis(2-hydroxypropyl)amine, N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine, and N-nitrosobis(2-oxopropyl)amine. Two other major metabolites have been detected, one which elutes near the solvent front and is referred to as X1, and one which is more hydrophobic than N-nitrosobis(2-oxopropyl)amine and is called X2. The rates of formation of these metabolites were linear with protein-up to a final concentration of 15 mg of pancreatic S-9 per ml. The *in vitro* metabolism of NNDM by liver and pancreas S-9 and microsomes was compared. The enzyme(s) for the initial oxidative metabolism of NNDM are associated with the microsomes in the liver and the pancreas. Pretreatment of hamsters with 2,3,7,8-tetrachlorodibenzo-p-dioxin results in a marked increase in the rate of NNDM metabolism by both acinar and islet cells, and in a greater than 20-fold induction of aryl hydrocarbon hydroxylase activity in acinar cell S-9. These results suggest the presence of different forms of the microsomal mixed-function oxidases in these two pancreatic cell types. Duct cells can also metabolize NNDM to an active form as demonstrated by unscheduled DNA synthesis in the nuclei of pancreatic duct epithelial cells after exposure to NNDM. These results provide additional evidence that pancreatic carcinogens are activated within the target cell(s) of the pancreas. (32 Refs)

108. EFFECT OF MICROSOMAL CYTOCHROME P-450 ISOENZYME INDUCTION ON THE MUTAGENIC ACTIVATION OF 2-AMINOANTHRACENE

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Two rabbit microsomal cytochrome P-450 isozymes, Forms 4 and 6, which are differentially induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the liver in an age-dependent fashion, catalyze the activation of 2-aminoanthracene to a mutagen in the Ames Salmonella mutagenesis assay. We have shown previously that in the presence of saturating concentrations of 2-aminoanthracene, Form 4 is 15-fold more active than Form 6 in the activation of this mutagen. Similar differences in the activation of 2-aminoanthracene are observed between liver microsomes isolated from TCDD-treated adult rabbits, in which Form 4 predominates, and microsomes from rabbit neonates exposed transplacentally to TCDD prior to birth, in which Form 6 predominates. However, when the extent of mutagenesis is limited by the amount of 2-aminoanthracene and is independent of the rate of activation, the number of revertants produced is similar for microsomes isolated from either newborn or adult TCDD-treated rabbits. Under these conditions, the extent of mutagenesis obtained for a given amount of 2-aminoanthracene will depend on the balance between activation and competing re-

action pathways leading to detoxication. Thus, differences in the rate of activation between adult and newborn microsomes are probably offset by similar differences in the rates of competing pathways of metabolism. This is consistent with the finding that the overall rate of 2-aminoanthracene metabolism by the adult microsomes is greater than that of the neonate. In order for the extent of mutagenesis to be independent of rate, the half-life of 2-aminoanthracene was seen to be less than approx 12 min. (Author abstract) (15 Refs)

109. EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND PHENOBARBITAL ON THE OCCURRENCE AND DISTRIBUTION OF FOUR CYTOCHROME P-450 ISOZYMES IN RABBIT KIDNEY, LUNG, AND LIVER

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Cancer Res. 42(4):1423-1432 1982

The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and of phenobarbital (PB) on the distribution and occurrence of four cytochrome P-450 isozymes, Forms 2, 3, 4, and 6, in the kidney, lung, and liver of adult male rabbits was investigated using immunofluorescence. In the kidney, Forms 2 and 3 were localized in the proximal tubules of both untreated and PB-treated animals, while antibodies to Forms 4 and 6 showed weak to negative staining. In TCDD-pretreated animals, Forms 4 and 6 appeared in the renal endothelium, in addition to staining the proximal tubular epithelium intensely. Form 2 was the only isoenzyme of those studied found to be present in the lungs of normal and PB-treated rabbits; it was also present in lungs of TCDD-pretreated rabbits. Form 3 was not detected in any of the rabbit lungs examined. Forms 4 and 6, while not apparent in the lungs of normal or PB-treated animals, were found in the lungs of TCDD-treated animals and also appeared in the endothelium of the pulmonary arteries and veins. All forms tested were present in control liver. The staining for Form 2 was intense in the livers of PB-pretreated animals, as was the staining for Forms 4 and 6 in the livers of TCDD-pretreated animals. Our results indicate that, while PB altered the intensity of staining for Form 2 in the liver and kidney, TCDD altered both the staining intensity and distribution of the isozymes in kidney, lung, and liver, producing, for example, a localization of Forms 4 and 6 in the endothelium of both the kidney and lung which was not seen in either untreated or PB-pretreated rabbits. (Author abstract) (48 Refs)

110. DIOXIN: INDUCTION OF XENOBIOTIC METABOLISM

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Fund Cancer Toxicol. 19(3):387-389 1981

The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on xenobiotic-metabolizing enzymes are reviewed and discussed. The potency and persistence of TCDD as an enzyme inducer is due to its chemical structure, its high affinity for binding to a specific liver cytosol protein, and its metabolism and pharmacokinetics. The possible induction of xenobiotic-metabolizing enzymes in humans exposed to TCDD and the possible existence of a relationship between the inductive and toxic effects of TCDD in man are considered to merit further investigation. (67 Refs)

111. METABOLISM OF BENZO(A)PYRENE IN RAT PROSTATE GLANDS FOLLOWING 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN EXPOSURE

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Carcinogenesis, 379:823-831 1981

Benzo(a)pyrene (BP) metabolism was studied in the prostate glands of adult Sprague-Dawley rats treated with the potent monooxygenase inducer 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 2.5, 5, or 10 µg/kg, po). TCDD not only caused selective induction of prostatic aryl hydrocarbon hydroxylase (AHH) and cytochrome P-448, but also resulted in a significant increase in organic-soluble BP metabolites over aqueous conjugated metabolites. High-pressure liquid chromatography analysis showed that formation rates of phenols, quinones, and dihydrodiols in TCDD-induced prostate glands were, respectively, 290, 192, and 38 times higher than in non-induced glands. The rate of 7,8-diol formation was 1.5 times that of 4,5-diol and 3 times that of 9,10 diol. TCDD-induced increases in BP metabolites were dose dependent, and they coincided with the magnitude of AHH induction. The possibility that increased 7,8-diol synthesis could lead to increased synthesis of the ultimate BP carcinogen and prostate tumor induction is discussed. (30 Refs)

112. POLYHALOGENATED BIPHENYLS AND PHENOBARBITAL: EVALUATION AS INDUCERS OF DRUG METABOLIZING ENZYMES IN THE SHEEPSHEAD, ARCHOSARGUS PROBATOCEPHALUS

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Chem Biol Interact, 36/37:229-248 1981

Several doses of Aroclor 1254 (polychlorinated biphenyl (PCB) mixture), Firemaster FFI (polybrominated biphenyl (PBB) mixture), 2,2',4,4',5,5'-hexabromobiphenyl (HBB), 3,3',4,4',5,5'-hexachlorobiphenyl (HCB) and phenobarbital (PB) were administered to the marine fish sheepshead (*Archosargus probatocephalus*). The PCB and PBB mixtures caused induction of hepatic microsomal benzo(a)pyrene hydroxylase (AHH), 7-ethoxycoumarin O-deethylase (7-EC) and 7-ethoxycoumarin O-deethylase (ERF) activities, but not benphetamine N-demethylase (BND), epoxide hydrolase (EH) or glutathione S-transferase (GSH-T) activities. This induction pattern is typical of that caused by polycyclic aromatic hydrocarbons (PAH) in fish and mammals or by tetrachlorodibenzo-p-dioxin (TCDD) in mammals. The extent of induction of AHH activity and cytochrome P-450 content was higher when experiments were carried out in summer (water temperature 25 ± 4°C) than in winter (water temperature 11 ± 3°C). Firemaster FFI (15 mg/kg) induced fish for at least 56 days in both summer and winter at which time the liver concentrations of PBB were in the ppm range. PCB concentrations in the ppm range have been found in fish from polluted lakes and seas, thus we may expect that environmental exposure to PCB is sufficient to induce hepatic mixed function oxidase (MFO) activities. The PCB isomer 3,3',4,4',5,5'-HCB, which induces the same spectrum of hepatic drug-metabolizing activities as TCDD and PAH in rats, had a broadly similar effect in the sheepshead. Another purified isomer 2,2',4,4',5,5'-HBB, which induces the same enzymes as PB in rats, had no effect on drug-metabolizing activities in sheepshead. PB was also without effect on sheepshead hepatic drug-metabolizing enzymes, although a typical narcotic effect was produced in PB-treated sheepshead. Our studies provide further evidence that drug-metabolizing activities in fish liver are readily induced by chemicals like TCDD or PAH, but

we fail to demonstrate induction after treatment of sheepshead with inducers of the PB type. (Author abstract) (40 Refs)

113. DIOXIN AND CANCER

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Biochem, 111:1:977-979 1980

The carcinogenicity of dioxin has been established in rats and mice, but not as yet in man. Dioxin apparently acts upon cellular DNA by forming a receptor complex, after the manner of steroid hormones. The consequence of the actions of the dioxin-receptor complex on chromosomal DNA is the formation of aryl hydrocarbon hydroxylases (AHH), which are much more effective than the monooxygenases of normal cells in generating toxic metabolites from environmental pollutants. Experiments in rats treated with benzo(a)pyrene (BP) and dioxin showed that dioxin is a strong cocarcinogen. Dioxin pretreatment markedly enhanced the binding of carcinogenic BP metabolites to liver cell DNA. In some animal strains, dioxin was a 30,000-fold stronger inducer of hepatic AHH than 3-methylcholanthrene and a 1-10 million-fold stronger inducer of cutaneous AHH than 7,12-dimethylbenzanthracene (no further details). It may be possible to prevent this induction of AHH by neutralizing cytochrome P-448 and P-450 activities. The results of laboratory experiments suggest that ellipicine may counteract the effects of AHH-inducing substances. (9 Refs)

114. PESTICIDES AS INDUCERS OF HEPATIC DRUG-METABOLIZING ENZYMES. II. GLUTATHIONE S-TRANSFERASES

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Gen Pharmacol, 11/3:437-441 1980

The changes in activity of hepatic glutathione S-transferases were assayed with three different substrates, after mice were exposed to pesticides and other xenobiotics. The compounds tested included 16 pesticides, 3-methylcholanthrene (MCA) phenobarbital (PB), chloral hydrate, two insecticide synergists (piperonyl butoxide and sesamex), and one cotton defoliant. The enzymes investigated were reduced glutathione S-aryl transferase (GSH-SAT), GSH S-epoxide transferase (GSH-ET), and GSH S-alkyl transferase (GSH-SART). ICR-Dublin strain mice were given no more than 100 mg/kg of each agent for 3 days ip or po. GSH-ET decreased slightly after treatment with malathion or etrimfos. No change was observed in any of the enzyme activities after treatment with 2,4-D, MCA, and trifluralin. Modest induction of at least one enzyme was noted with several compounds. More than 50% induction was noted with CGA-19255, 2,4,5-T, TH-6040, Gardona, and endosulfan. TH-6040 caused more than a 2-fold increase in the activities of GSH-SAT and GSH-SART. PB induced up to a 4-fold increase in GSH-SAT and a 2-fold increase in the other two enzymes. These results suggest that these enzymes are inducible and that mice appear to be more responsive than rats. The inductive effects of these compounds are dose dependent, vary with the route of administration and are selective with regard to different GSH S-transferase activities. (21 Refs)

115. GENETIC EXPRESSION OF AFLATOXIN B1 METABOLISM: EFFECTS OF 3-METHYLCHOLANTHRENE AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON THE METABOLISM OF AFLATOXINS B1 AND B2 BY VARIOUS INBRED STRAINS OF MICE

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Mol Pharmacol; 10(2):296-303 1980

The hepatic microsomal metabolism of aflatoxin B1 and aflatoxin B2 following pretreatment with phenobarbital (PB), 3-methylcholanthrene (MC), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was examined in several strains of mice. The mice were of two types, those with aryl hydrocarbon hydroxylase (AHH) activity, Ah responsive and those that were lacking AHH activity, Ah nonresponsive. PB pretreatment induced aflatoxin B1 2,3-epoxide (AFB12) activity and aflatoxin B1 4-hydroxylase (AFB19) activity but did not induce aflatoxin B1 4-hydroxylase (AFB14) activity indicating that AFB12 and AFB19 activities are catalytically induced by cytochrome P-450. Pretreatment with MC had no effect on AFB12 or AFB19, but did induce AFB14 and aflatoxin B2 4-hydroxylase (AFB24) in Ah responsive strains. This suggested that the AFB14, AFB24 and AHH are controlled by the same genetic factors. Induction of AHH activity by TCDD was dose-dependent: in both Ah responsive and Ah nonresponsive strains. AFB12 and AFB19 activity in Ah nonresponsive strains was increased by 20-25% with 9 x the dose required to induce these enzymes in the Ah responsive strain. It was concluded that the different P-450's which metabolize aflatoxin via the various pathways are under different regulatory control and that closely linked genetic factors at the regulatory level control the induction of AFB14, AFB24 and AHH. (30 Refs)

116. INDUCTION OF ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN THE RAT PROSTATE GLANDS BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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J Pharmacol Exp Ther; 215(1):601-605 1980

A single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 10 ug/kg, po) caused a significant increase in aryl hydrocarbon hydroxylase (AHH) and cytochrome P-450 levels in the prostate glands of adult CD rats. By 16 hr after TCDD administration, the AHH activity was 200% the control value, and it remained elevated for 3 wk. Pretreatment with actinomycin D or cycloheximide inhibited the AHH and cytochrome P-450 induction. (37 Refs)

117. METABOLISM OF BENZO(A)PYRENE BY THE ISOLATED PERFUSED RAT TESTIS

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Cancer Res; 40(9):3297-3303 1980

Benzo(a)pyrene (BP) metabolism was studied in control and tetrachlorodibenzo-p-dioxin (TCDD)-induced rat testes using the isolated perfusion technique. The testicular artery was perfused with [7,10-¹⁴C]BP (specific activity, 60.7 mCi/mmol) dissolved in a Krebs-Ringer bicarbonate buffer fortified with 3% bovine serum albumin (Fraction V) and equilibrated with 95% O₂ and 5% CO₂ for 20 min at 32 C. The BP concentration was 2.8×10^{-7} M, and the rate of perfusion was 20 ml/hr/g testis. BP metabolites were extracted

from both the perfusate and the testicular tissue and subjected to high-pressure liquid chromatographic analysis. Comparison of BP metabolites in control and TCDD-induced testes showed that the total amounts of BP metabolites recovered during the 60-min perfusion were 12.3 and 26.2% of total radioactivity, respectively. The pattern of BP metabolites in the control testes demonstrated that the major metabolites in the organic extractable phase were 9,10-dihydroxy-9,10-dihydrobenzo(a)pyrene (52.1%) and 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene (16.3%). Smaller amounts of 3-hydroxybenzo(a)pyrene (9.2%), quinones (8.6%), and 4,5-dihydroxy-4,5-dihydrobenzo(a)pyrene (4.4%) were also present. This pattern of BP metabolites contrasts with that observed in rat livers or lungs, where phenols and quinones are the primary metabolites. TCDD increased BP metabolism with a concomitant increase in the testicular aryl hydrocarbon hydroxylase activity. The magnitude of various BP metabolites in TCDD-treated rats ranged from 1.5 to 2.8 x control, except 4,5-dihydroxy-4,5-dihydrobenzo(a)pyrene which was not altered by TCDD treatment. Thus, mixed-function oxidase(s) in rat testis preferentially epoxidize the 9,10 and 7,8 positions of BP. Since 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene is the precursor of the potent carcinogen, 1,7+8-dihydroxy-9,10-oxo-7,8,9,10-tetrahydrobenzo(a)pyrene, the TCDD-induced shift in metabolic products could be significant in BP-induced mutagenic and/or carcinogenic effects. (Author abstract) (34 Refs)

118. CATALYSIS OF DIVERGENT PATHWAYS OF 2-ACETYLAMINOFLUORENE METABOLISM BY MULTIPLE FORMS OF CYTOCHROME P-450

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Cancer Res; 40(12):4456-4459 1980

Four highly purified forms of rabbit hepatic, microsomal cytochrome P-450 catalyze the N- and ring-hydroxylation of 2-acetylaminofluorene (AAF) at different rates. Form 4, the major form of the cytochrome induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in adult rabbit liver, catalyzed the N-hydroxylation of AAF more rapidly than did the other three forms. N-Hydroxy-2-acetylaminofluorene accounted for 70% of the metabolites formed by the action of this cytochrome. Form 6, the major form of the cytochrome induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in neonate rabbit liver, and Form 3, a constitutive form of the cytochrome, both metabolized AAF at one-half the rate observed for Form 4. Phenols accounted for more than 90% of the metabolites produced by these two cytochromes. The major phenobarbital-inducible cytochrome P-450, Form 2, exhibited practically no catalytic activity (less than 1% of the other forms) with AAF as a substrate. Since N- and ring-hydroxylation are thought to represent divergent pathways of carcinogen metabolism (activation vs detoxification), the differential occurrence of the various cytochrome forms should affect the balance between these two reaction pathways. In this sense, cytochrome P-450 induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin differentially affects the magnitude and direction of in vitro microsomal metabolism of AAF as a function of age. (Author abstract) (28 Refs)

119. GONADAL ARYL HYDROCARBON HYDROXYLASE IN RATS AND MICE

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Cancer Res. 38(5):1368-1373 1978

Rat and mouse gonadal aryl hydrocarbon hydroxylase (AHH) activity was measured and characterized in control and 3-methylcholanthrene (3-MC: 80 mg/kg, ip)-treated animals. The ovarian AHH activity increased two- to threefold in Sprague-Dawley, Lewis, and Brown-Norway rats following 3-MC treatment. The same treatment decreased the testicular AHH activity in Sprague-Dawley rats. The induction of AHH activity after 3-MC administration was the same in ovaries from weanling and adult Sprague-Dawley rats. Ovarian AHH activity was localized in the microsomal fraction, required NADPH, and had a broad pH optimum between 7.0 and 8.8. Both CO and antibody to cytochrome c reductase inhibited ovarian AHH activity, suggesting that the hydroxylase is a cytochrome P-450-dependent monooxygenase. Basal testicular and ovarian AHH activity was similar in C57BL/6N (B6) and DBA/2N mice; 3-MC treatment, however, increased gonadal AHH activity only in B6 mice. Treatment with 50 ug/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin resulted in an increase in testicular AHH activity in both B6 and DBA/2N mice. Seven days after a single 3-MC injection, 87% of the primordial oocytes were destroyed in B6 mice, 69% were destroyed in DBA/2N mice, and none were destroyed in Sprague-Dawley rats. These findings suggest that the oocyte toxicity of 3-MC in mice is related to ovarian AHH activity; this association is absent in rats. This system may be useful for the study of environmentally induced ovarian cancer in humans. (27 Refs)

C.

OTHER METABOLIC EFFECTS

120. A 60 KD ONCOFETAL PROTEIN INDUCTION IN RATS BY CHEMICAL CARCINOGENS (MEETING ABSTRACT)

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Fed Proc. 44(4):952 1983

A 60 kilodalton oncofetal protein is released to circulation in vivo from tumor cells, or from tissues following carcinogen treatment (Cancer Lett 20: 277, 1983). In the present study the oncofetal protein has been evaluated against single dose regimens of 17 known carcinogens and 9 known non-carcinogens from various structural groups such as polycyclic aromatic hydrocarbons, aromatic amines, nitrosamines, fungal toxins and dioxins. The chemical compounds were administered ip to 50 day old female Sprague Dawley rats. The biochemical assay involved molecular sieving on Sepharose CL-6B of the plasma proteins isolated from the blood 21 days post-treatment with the test chemicals, followed by a measurement of specific nuclear RNA releasing activity in the 60 kd fraction. All 17 carcinogens tested induced the 60 kd oncofetal protein, as measured by its messenger RNA transport activity. In contrast, none of the nine non-carcinogens tested, including non-carcinogenic analogs and non-carcinogenic toxins, induced the oncofetal protein. The induction of reversible phenotypic changes in normal neonatal cells by tumor promoters did not induce the 60 kd factor. These results strongly suggest that the induction of the 60 kd oncofetal protein is specific for carcinogens. (1 Ref)

121. THE ROLES OF GLUTATHIONE PEROXIDASE AND HYDROGEN PEROXIDE IN TCDD-INDUCED LIPID PEROXIDATION (MEETING ABSTRACT)

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Proc. 1st International Symposium on Biological Reactive Intermediates, June 6-8, 1983, College Park, MD, abstract 49, 1983.

Previous studies have shown that TCDD induces hepatic lipid peroxidation and inhibits selenium dependent glutathione peroxidase activity (GSH-Px). Lipid peroxidation may contribute to the progressive wt loss and general debilitation which occurs following TCDD administration and may explain the lack of tissue specificity with respect to the toxic effects of this xenobiotic. Initial studies were based on the use of high doses of TCDD. We have examined the dose and time dependent effects of TCDD on hepatic lipid peroxidation (malondialdehyde content) and GSH-Px activity and the ability to induce microsomal malondialdehyde production in hepatic and extrahepatic tissues of male and female rats. The results indicate that a dose of TCDD as low as 1 ug/kg can induce hepatic lipid peroxidation and inhibit GSH-Px. Furthermore, lipid peroxidation occurs in extrahepatic tissues as kidney, thymus and heart. Differences occur in onset and rates of lipid peroxidation between male and female rats. The addition of catalase and free radical scavengers to microsomes markedly inhibits malondialdehyde formation. Thus, hydrogen peroxide and free radical production by microsomes in conjunction with a decrease in GSH-Px activity may facilitate TCDD induced lipid peroxidation.

122. INDUCTION OF SISTER CHROMATID EXCHANGES BY THE PEROXISOME PROLIFERATORS 2,4-D, MCPA, AND CLOFIBRATE IN VIVO AND IN VITRO

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Carcinogenesis 31(6):703-7 1984

Phenoxy acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) have been found to induce proliferation of peroxisomes in the liver cells of rodents in the same manner as the hypolipidemic drug clofibrate. Both phenoxy acid herbicides and clofibrate (ethyl-alpha-p-chlorophenoxyisobutyrate) are suspected carcinogens. The present study reports the effects of these agents on the induction of sister chromatid exchange (SCE) in the blood lymphocytes of exposed rats (100 mg/kg with 2,4-D and MCPA, 200 mg/kg with clofibrate for 2 weeks in one intragastric dose/day), in the bone marrow cells of exposed Chinese hamsters (100 mg/kg, treatments as above), and in Chinese hamster ovary (CHO) cells in vitro (10(-5), 10(-4), and 10(-3) M. for 1 h). In the experiments in vitro, the effects of purified 2,4-D and MCPA phenoxy acids were studied, in addition to those of the commercial herbicide formulations and clofibrate. No increase of SCE frequency was observed in the blood lymphocytes of the exposed rats in comparison with the controls. In the bone marrow cells of the exposed Chinese hamsters, a slight increase of SCE was found in the group treated with MCPA but not in the groups treated with 2,4-D or clofibrate. A slight increase in the number of SCEs was characteristic of all the treated CHO cell cultures, both with and without a rat liver microsomal activation system (S9 mix). No clear dose-related effects, however, could be discerned with any of the compounds, and no differences in the SCE induction were observed between the commercial herbicide products and the purified phenoxy acetic acids. The present results support the data which indicate that 2,4-D, MCPA, and clofibrate do not act as direct DNA-damaging agents.

123. PESTICIDE-INDUCED COMPLETE AND PARTIAL CHROMOSOME LOSS IN SCREENS WITH REPAIR-DEFECTIVE FEMALES OF *DROSOPHILA MELANOGASTER*

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Environ Manage; 5(6):835-46 1983

Commercial preparations of fungicides (captan and maneb), herbicides (bromacil, paraquat, picloram, and 2,4-D), and insecticides (carbaryl, chlorpyrifos, dimethoate, DDT, diazinon, carbofuran, and permethrin) were tested for their ability to induce complete and partial chromosome losses in *Drosophila melanogaster* males. In an attempt to identify the mutagenic activity of pesticides that are toxic in low concentrations in *Drosophila*, these males were mated with *mus-302* repair-defective females. The rationale for this mating scheme is based on the repair of genetic damage in *Drosophila* sperm by maternal enzymes in the zygote, and on the reports that there may be increases in the frequency of recovery of chemically induced chromosome losses in crosses of treated males with *mus-302* females. Verification of the sensitivity of this screen in this study came from significant increases in the frequency of chromosome loss induced by low concentrations of the positive controls, N-nitrosodimethylamine and methyl methanesulfonate. Of the 13 pesticides, the insecticide chlorpyrifos induced a significant amount of ring-X chromosome loss. No pesticide induced a significant increase in partial chromosome loss. These results are discussed in relation to the usefulness of repair-defective mutants in screens for genetic damage in *Drosophila* and other higher eukaryotes by chemicals that are toxic or cause sterility at low concentrations.

124. DIFFERENTIAL EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON NUCLEAR RNA POLYMERASE ACTIVITY IN THE RAT LIVER AND THYMUS

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Biochem Pharmacol; 31(15):2459-2462 1982

An increase in RNA polymerase B activity in hepatic cell nuclei was observed after a single intravenous injection of 1 µg/rat of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The enzyme activity was about 70% above the control value at 1 hr after injection and then declined rapidly. A secondary increase was evident at 24 hr. Prior to the secondary increase in RNA polymerase B activity, there was an increase in RNA polymerase A activity which was about 125% above the control value. The initial increase in RNA polymerase B activity was sensitive to both alpha-amanitin and actinomycin D injected 30 min before TCDD administration. In the thymus, an increase in RNA polymerase B activity was observed 4 hr after injection (25% above control value) but thereafter it declined and at 24 hr it was about 30% below the control value. RNA polymerase A activity was inhibited as early as 1 hr after injection but had returned to the control value at 4 hr and then paralleled RNA polymerase B activity. Thus, TCDD stimulated RNA synthesis in the rat liver but the drug inhibited RNA synthesis in the rat thymus. Since these effects were detected as early as 1 hr after TCDD administration it is proposed that TCDD action requires transcriptional response and that this response may represent a primary site of TCDD action in the cell. The cellular entities or mechanisms which TCDD uses to alter transcription in the cell nucleus remain to be determined. (Author abstract) (30 Refs)

125. PHENOXY ACID HERBICIDES CAUSE PEROXISOME PROLIFERATION IN CHINESE HAMSTERS

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Scand J Work Environ Health; 8(1):70-73 1982

Chinese hamsters 12-20 weeks old were given by gavage saline dilutions of commercial herbicides containing either 2,4-dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MCPA). No other active ingredient was present. Nine daily doses were given at 100 mg/kg. The positive control group received clofibrate (CF; 100 mg/kg/day sc, x 7). All test and control animals were sacrificed 1 day after the last dose. Peroxisomes were chemically localized in liver samples and the areas examined by electron microscopic projection. MCPA and CF significantly increased the size of the peroxisomes (p less than 0.001). CF and 2,4-D also increased significantly the number of peroxisomes (p less than 0.001). CF is chemically related to the two herbicides and has been suggested to exert its carcinogenic action through excess production and/or breakdown of hydrogen peroxide resulting in DNA damage. It is suggested that the increased incidence of soft tissue sarcoma and malignant lymphoma related to exposure to phenoxy acid herbicides may derive from the same mechanism, since both herbicides tested increase the size of or proliferate peroxisomes. (11 Refs)

126. 2,4-DICHLOROPHENOXYACETIC ACID - A NEW ENVIRONMENTAL MUTAGEN

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Nature Res; 105(2):111-114 1982

The mutagenic and teratogenic potential of 2,4-dichlorophenoxyacetic acid (2,4-D) were tested on barley (*Hordeum vulgare*) seeds at a concentration of 100 ppm. The compound did not affect markedly seedling height, but reduced germination and seed set. Other effects on the M1 generation included increased chromosomal aberrations, increased chlorophyll chimera, and increased aberrant pollen mother cells and sterile pollen. In the M2 generation the chlorophyll-deficient mutations albina, viridis, chlorina, ligula, striata, albiviridis, and viridialba occurred, with ligula types dominant (58-61%). While the mutagenic properties of 2,4-D demonstrated are potentially useful to breeders, caution is suggested in its use as an herbicide or hormone. (15 Refs)

127. ALLERGIC HYPERSENSITIVITY TO THE HERBICIDE 2,4-D IN BALB/C MICE

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J Toxicol Environ Health; 10(4-5):729-41 1982

Following several reports of human allergic hypersensitivity, the ability of 2,4-D (2,4-dichlorophenoxyacetic acid) to elicit 2,4-D-specific IgE antibodies and delayed-type hypersensitivity in BALB/c mice was studied. 2,4-D-specific IgE antibodies were detected in mouse sera following the second intraperitoneal immunization with 1, 10, or 100 micrograms 2,4-D-keyhole limpet hemocyanin conjugate with aluminum hydroxide adjuvant. Specific IgE was determined with the rat passive cutaneous anaphylaxis test using a conjugate of 2,4-D with bovine serum albumin for challenge. The highest antibody titers and a measurable response in all mice were seen in the group that received 1 microgram of 2,4-D conjugate. Dinitrophenyl-specific IgE was measured at all intervals examined in mice immunized with a dinitrophenyl-keyhole limpet hemocyanin conjugate. 2,4-D applied epicutaneously

on 2 d or over 4 wk failed to elicit delayed-type hypersensitivity as measured by change in ear thickness, incorporation of 5-[125I]iodo-2'-deoxyuridine, or histology following challenge on the ear. No 2,4-D-specific IgE antibodies were detected in serum during the 4-wk sensitization period. Similar treatment with a known sensitizer, dinitrofluorobenzene, produced delayed hypersensitivity. Following 4 wk of dosing, low titers of dinitrophenyl-specific IgE antibodies were elicited.

128. A UNIQUE CHANGE OF STEROID METABOLISM IN RAT LIVER MICROSOMES INDUCED WITH HIGHLY TOXIC POLYCHLORINATED BIPHENYL (PCB) AND POLYCHLORINATED DIBENZOFURAN (PCDF)

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Inst. Pharmaceutical Science, Hiroshima Univ. Sch. Medicine, Hiroshima 734, Japan

J Pharmacol Dyn. 5:121-994-1004 1982

Pretreatment of rats with highly toxic polychlorinated biphenyl and polychlorinated dibenzofurans (which are potent 3-methylcholanthrene-type inducers) increased selectively 2 α -hydroxylation, but strongly suppressed, 2 α -, 6 β - and 16 α -hydroxylation as well as 2 α -reduction of progesterone and testosterone in liver microsomes. Results suggest that this unique change of steroid metabolism produced by the toxic agents may be responsible, at least in part, for the endocrine symptoms caused by these compounds via disturbance of steroid homeostasis. (39 Refs)

129. MUTAGENICITY STUDIES WITH 2,4,5-T ON BACTERIA AND MAMMALIAN GERM CELLS

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Toxicology: Carcinog. Mutagen. 21:151-161 1982

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was tested for mutagenicity in an Ames test with microsome activation, a dominant lethal test in rats, and a cytogenetic assay of Chinese hamster spermatogonia. The 2,4,5-T contained 0.05 ppm dioxin. *Salmonella* strains used were TA 98, 100, 1535, and 1537, with cyclophosphamide and tryptophan as controls. 2,4,5-T was tested at 4-2,500 μ g/plate. In the dominant lethal test rats were: 0, 1, or 100 mg/kg 2,4,5-T for 8 wk and then mated. Corpora lutea, dead, and viable embryos were counted 12 days after mating, and the rate of dominant lethal mutations determined. For the cytogenetic assay male Chinese hamsters were fed 0, 0.5, 5, or 50 mg/kg 2,4,5-T per day for 5 days in one test, and 0 and 500 mg/kg on the same schedule in a second test. The 2,4,5-T was not mutagenic in any of these tests. These findings are compared with tests of 2,4,5-T in other systems. (46 Refs)

130. THE MECHANISM OF BENZODIARONE ACTIVITY

Pizzavini M, Aho MF, Marsilongo R, Nazzari E

Istituto di Chimica Biologica, Università, Roma

Quart. S. Pietro Delle, 10121-205-8 1982

Keywords (MeSH): Allopurinol/pharmacodynamics; Animal; Benzodiarone/*pharmacodynamics; Benzofurans/*pharmacodynamics; Rat; Rat, Inbred Strains; Xanthine Oxidase/*antagonists and inhibitors/metabolism.

131. ENHANCED LIVER DNA SYNTHESIS IN PARTIALLY HEPATECTOMIZED RATS PRETREATED WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Toxicol Appl Pharmacol. 58(3):389-398 1981

The response of hepatocytes to a proliferative signal was studied in rats treated with 2,3,7,8-tetrachlorodibenzo-p-

dioxin (TCDD). Rats underwent a 1/3 hepatectomy 5 days after treatment with TCDD or acetone/corn oil (controls). Incorporation of 3H-thymidine (3H-TdR) into hepatic DNA in response to hepatectomy was 8-10% greater in TCDD-treated rats than in controls. The TCDD-enhanced 3H-TdR incorporation did not occur until after a latency period characteristic of regenerative DNA synthesis. (45 Refs)

132. EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON THE HEPATIC STORAGE OF RETINOL IN RATS WITH DIFFERENT DIETARY SUPPLIES OF VITAMIN A (RETINOL)

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Arch Toxicol (Berl). 45(4):275-283 1980

The effect of various dietary sources of vitamin A on liver storage of retinol has been investigated in Sprague-Dawley rats treated with single oral doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): 0, 0.1, 1.0, or 10 μ g/kg. Each dose group consisted of three subgroups, each comprising 10 rats which received a diet with normal, low or high retinol content. The animals were killed 4 wk after TCDD administration. Analyses of retinol were performed by high pressure liquid chromatography and glucuronosyltransferase activities were determined spectrophotometrically. A dose-dependent decrease in hepatic storage of retinol was evident. The high retinol diet did not fully compensate for the reduction caused by the highest TCDD-dose. Glucuronosyltransferase activity increased directly in relation to the TCDD-dose but in inverse proportion to the retinol content of the diet. (Author abstract) (58 Refs)

133. MUTAGENICITY OF URINE FROM RATS AFTER ADMINISTRATION OF 2,4-DIAMINOANISOLE: THE EFFECT OF MICROBOMAL ENZYME INDUCERS

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Mutat Res. 79(4):507-517 1980

Phenobarbital (PB)-pretreatment of rats induced a two-fold increase in the revertant rate of 24-hr urine tested in the Ames *Salmonella* system. Pretreatment with beta-naphthoflavone, 3-methylcholanthrene, or 2,3,7,8-tetrachlorodibenzo-p-dioxin, on the other hand, decreased the mutagenicity of 24-hr urine by about 70% in the presence of an activating system. (12 Refs)

D.

MODULATION OF TOXIC AND METABOLIC EFFECTS

134. EFFECT OF THYROID HORMONES ON LIVER MICROSOMAL ENZYME INDUCTION IN RATS EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Toxicology. 37(1-2):51-65 1983

The effect of thyroidectomy and thyroid hormone replacement therapy on liver microsomal enzyme induction was studied in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats (100 micrograms/kg). Treatment of non-thyroidectomized rats with TCDD had no effect on the concentration of liver microsomal cytochrome b5. In contrast, cytochrome b5 content was increased by TCDD treatment of thyroidectomized rats, regardless of replacement therapy with either T3 or T4. TCDD treatment increased the concentration of cy-

sochrome P-450 (2-3-fold) and the activities of benzo(a)pyrene hydroxylase (4-7-fold), ethoxycoumarin O-deethylase (50-70-fold) and UDP-glucuronosyltransferase (5-7-fold) in non-thyroidectomized and thyroidectomized as well as thyroidectomized thyroid hormone treated rats; indicating the induction of these liver microsomal enzyme activities is independent of thyroid status. Because thyroid status alters the toxicity of TCDD but does not alter the ability of TCDD to induce microsomal enzymes, it appears that TCDD toxicity may not be directly related to microsomal enzyme induction.

135. A POSSIBLE ROLE OF LIPID PEROXIDATION IN TCDD TOXICITY.

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Diss Abstr Int 1986; 45(1):942-B 1986

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potentially toxic environmental pollutant. The biochemical lesions associated with the toxicity of this compound are not known. This study was undertaken to assess the hypothesis that the toxicity of TCDD may be caused in part by free-radical mediated lipid peroxidation. Initial results demonstrated that TCDD does induce hepatic lipid peroxidation as measured by the thiobarbituric acid and conjugated dienes assay methods. Therefore, the effects of TCDD administration on the following related parameters were also examined: hepatic glutathione peroxidase (GSPX), aryl hydrocarbon hydroxylase (AHH), glutathione S-transferase (GST), glutathione reductase (GSR) activities and reduced glutathione (GSH) levels. In rats TCDD induced AHH, GSR, and GST activities, and decreased GSH content. In the hamster, a highly resistant species to TCDD, neither an increase in lipid peroxidation nor a change in the activities of the above enzymes was observed. Guinea pigs are a highly sensitive species to TCDD and increased lipid peroxidation with no GSPX activity was detected. AHH activity was induced. Daily treatment of rats with butylated hydroxyanisole protected against TCDD toxicity, which was associated with reduced lipid peroxidation, increased GSPX activity, increased GSH levels, and decreased AHH activity. Vitamin A treatment in conjunction with TCDD elevated GSPX activity, enhanced AHH activity, and provided partial protection against a lethal dose of TCDD. Neither the administration of glutathione or vitamin E was able to protect against TCDD toxicity. GSPX activity is dependent on selenium. Rats were maintained on synthetic diets supplemented with: 0, 0.10 and 2.0 ppm selenium. Greatest GSPX activity was found in the 0.10 ppm selenium supplemented group. A lethal dose of TCDD in rats fed these 3 diets resulted in 0, 47 and 8% survival, respectively. GSPX may play an important role in protecting against TCDD-induced lipid peroxidation. Experiments conducted with free radical scavengers suggest that hydrogen peroxide and possibly singlet oxygen are involved in TCDD-induced hepatic microsomal lipid peroxidation. The results support the hypothesis that the toxicity of TCDD may be due in part to lipid peroxidation. (Author abstract)

136. INHIBITION IN VIVO OF THE FORMATION OF ADDUCTS BETWEEN METABOLITES OF BENZO(A)PYRENE AND DNA BY ARYL HYDROCARBON HYDROXYLASE INDUCERS

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Cancer Res. 41(19,part1):3453-3460 1981

The lungs of A/HeJ mice are susceptible to benzo(a)pyrene (BP)-induced neoplasia whereas the livers are resistant. Following po administration of a carcinogenic dose of [³H]BP,

radioactivity was associated with the DNA of both lung and liver. Analysis of the deoxyribonucleosides by high-pressure liquid chromatography showed that the major adduct in both tissues chromatographed as the (+)-7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDEI)-deoxyguanosine adduct. The (+)-7beta,8alpha-dihydroxy-9beta,10beta-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDEII)-deoxyguanosine adduct was 9 to 15% of the BPDEI adduct in both lung and liver. Although total DNA-associated radioactivity was approx 12-fold higher in liver than in lung, the specific activities of the BP diol-epoxide adducts were approx the same in these organs. Treatment of animals with beta-naphthoflavone (betaNF), an inhibitor of pulmonary adenoma formation, markedly decreased the amount of the BPDEI and BPDEII adducts in the lung and the liver. The decrease in the amount of these adducts in the lung correlates with the inhibition of tumorigenesis by betaNF. The inhibition of total DNA-associated radioactivity was significantly less than the BP diol-epoxide adducts. Thus, betaNF appears to inhibit BP-induced pulmonary neoplasia by reducing the amount of the BPDEI-deoxyguanosine adduct. Other inducers of aryl hydrocarbon hydroxylase were also tested for their effect on the formation of BP-deoxyribonucleoside adducts. Both 2,3,7,8-tetrachlorodibenzo-p-dioxin and Aroclor 1254 significantly reduced the amount of the BPDEI adduct in both lung and liver. These data would suggest that both 2,3,7,8-tetrachlorodibenzo-p-dioxin and Aroclor 1254, like betaNF, should protect against BP-induced pulmonary neoplasia. The effects of aryl hydrocarbon hydroxylase inducers on the binding of BP to DNA in vivo markedly contrast with the effect in vitro. Treatment of animals with aryl hydrocarbon hydroxylase inducers stimulates the formation of BP diol-epoxide adducts in vitro. The reason for the differences between our in vivo results and those predicted from in vitro studies is unclear. (Author abstract) (70 Refs)

137. EFFECTS OF BUTYLATED HYDROXYANISOLE ON THE ARYL HYDROCARBON HYDROXYLASE OF RATS AND MICE

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Chem Biol Interact. 37(3):337-350 1981

The effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on the aryl hydrocarbon hydroxylase (AHH) activities in the liver, lung and skin of rats and mice have been studied to examine the possible mechanisms of the anticarcinogenic actions of these compounds. Both compounds inhibit the hydroxylase activities of hepatic microsomes and nuclei, with BHA a more potent inhibitor than BHT. The AHH of lung microsomes is inhibited to a lesser extent by BHA and BHT than that of the liver. The AHH activities of both liver and lung microsomes become less susceptible to the inhibition after pretreatment of the animals with 3-methylcholanthrene (MC) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) but phenobarbital (PB) pretreatment does not produce such an effect. In skin homogenates, however, the AHH activities of control rats and mice are not inhibited by BHA and BHT. The only skin sample which is inhibited by BHA and BHT is that from TCDD-pretreated mice. It has been established that the extent of inhibition with different samples is related to the concentration of BHA in the incubation but not to the amounts or specific activities of microsomes used. Double reciprocal plots suggest that BHA exerts a mixed inhibition on the hydroxylase of liver microsomes with K_i of 7.7 μ M. Analysis of the metabo-

lines of benzo[a]pyrene (BP) shows that BHA inhibits the formation of various metabolites uniformly without changing the regioselectivity of the enzyme system. The mechanism of inhibition has also been studied with a reconstituted AHH system consisting of cytochrome P-450 (P-450), reductase and phospholipid. The system with P-450 isolated from PB-induced microsomes is inhibited to a much greater extent than that with MC-induced P-450. The results indicate that the inhibitory action of BHA is dependent on the species of the animal, tissue types and treatment with inducers. (Author abstract) (42 Refs)

138. INFLUENCE OF SOLVENTS AND ADSORBENTS ON DERMAL AND INTESTINAL ABSORPTION OF TCDD

Pogor H, Schlatter C

Inst. Toxicology, Federal Inst. Technology and Univ. Zurich, Zurich, Switzerland

Food Cosmet Toxicol. 18(5):477-481 1980

The effects of solvents and adsorbents on the uptake of ³H-labeled 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) after po administration to female Sprague-Dawley rats and after dermal administration to hairless rats were studied by measuring the liver concentration of TCDD. Activated carbon almost completely prevented intestinal TCDD uptake. Absorption of TCDD from soil mixtures was considerably lower than after po administration of an ethanolic soln of TCDD. After dermal application of TCDD, the radioactivity level in the liver was highest when TCDD was applied in methanolic soln and amounted to approx 50% of that after po administration. Adsorption of TCDD onto activated carbon completely prevented its percutaneous uptake. When TCDD was administered with oil, reproducible quantities in the liver were measured only after the administration of at least 50 µCi of TCDD/animal. Application of TCDD in a lipophilic ointment (vaseline) also yielded low liver levels. With polyethylene glycol 1500 formulations, incorporation of 15% water into the ointment base caused a marked increase in TCDD uptake. Similar formulations of TCDD were also tested for their ability to produce chloracne in the rabbit ear. Threshold levels of TCDD for chloracne induction were 1 µg/ear for pure TCDD, 160 µg/ear for TCDD adsorbed onto activated carbon, and 1 µg/ear for vaseline and polyethylene glycol preparations of TCDD. (12 Refs)

139. IRON DEFICIENCY PREVENTS LIVER TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

Summery GD, Jones RG, Cole PM, Buford D, Krestynski F

Dept. Medicine, McMaster Univ., Hamilton, Ontario L8S 4J9, Canada

Scancer. 204(4900):332-335 1979

Comparison of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD: 25 µg/kg/wk ip, for 11 wk) in iron-deficient and normal C57BL/6J mice demonstrated that iron deficiency prevented TCDD-induced hepatocellular damage, porphyria, and other toxic effects. (18 Refs)

E.

PHARMOKINETICS

140. IDENTIFICATION OF METABOLITES OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) FORMED ON INCUBATION WITH ISOLATED RAT HEPATOCYTES

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Chemical Industry Inst. Technology, Research Triangle Park, NC, 27709

Biochem Biophys Res Commun. 105(1):341-346 1982

The metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by isolated rat hepatocytes was investigated. Metha-

nol extracts of the incubation mixture were analyzed by high performance liquid chromatography (HPLC). Fractions corresponding to TCDD metabolites were subjected to hydrolysis by beta-glucuronidase, derivatized with diazomethane, and analyzed again by HPLC followed by gas chromatography-mass spectrometry. Comparison with authentic methoxy derivatives of TCDD revealed two TCDD metabolites: 1-hydroxy-2,3,7,8-tetrachlorodibenzo-p-dioxin and 8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin. (11 Refs)

141. STRUCTURE ELUCIDATION OF MAMMALIAN TCDD-METABOLITES

Pogor H, Bauer HR, Weber H, Zwerfel U, Schlatter C

Inst. Toxicology, Federal Inst. Technology and Univ. Zurich, Schorenstr. 16, CH-8063 Schwerzenbach, Switzerland

Experientia. 38(4):484-486 1982

Thin layer and gas chromatographic examination of the bile of dogs which were given tritium-labeled 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) revealed the presence of several polar biotransformation products. The structure of 5 phenolic metabolites was elucidated by combined gas chromatography-mass spectrometry. A metabolic scheme for TCDD in the dog is proposed. (Author abstract) (13 Refs)

142. NEW PATHWAYS FOR CHLORINATED DIOXINS

Hay A

Dept. Chemical Pathology, Univ. Leeds, Leeds, England

Nature. 290(5841):514-515 1981

Although 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is itself active, approx 1% of TCDD is metabolized. TCDD metabolism has been observed in vitro in Golden Syrian hamster hepatocytes. A reaction scheme for the metabolism of TCDD in rats and dogs is presented. Dynamic equilibrium between adipose and other tissues to maintain blood TCDD levels has been postulated for some animals. In humans, the TCDD blood levels are low but long-lasting. The metabolism of other polychlorinated compounds is discussed with reference to the Yusho I and II cases. Carcinogenic risk associated with TCDD is discussed. (no Refs)

143. FATE OF 2,3,7,8-TETRACHLORODIBENZOFURAN IN THE MONKEY

Bordas LS, Doud GM, Matthews HB, McConnell FE

Toxicol Appl Pharmacol. 57(2):189-196 1981

IV.

IN VITRO STUDIES ON DIOXINS AND DIBENZOFURANS

A.

STUDIES OF GENOTOXIC EFFECTS

144. INCREASED RATES OF SISTER CHROMATID EXCHANGES INDUCED BY THE HERBICIDE 2,4-D

Torkala TE, Jalil SM

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J Hered. 70(3):213-4 1981

The potential for genetic damage from widely used herbicide herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D), continues to be of serious concern. The mutagenic effect as reflected by the rates of sister chromatid exchanges (SCE) was determined in cultured human lymphocytes. Data were based on the analysis of 50 cells for the control and each of the three treatments. A 50 micrograms/ml dosage caused a highly significant increase in SCE. Dosages of 100 and 250

micrograms/ml elevated the rate of SCE, but not significantly. Since 2,4-D biodegrades rapidly in soil and water, its continued use is not in serious question until safer compounds are available. However, the results of this study suggest that the danger of genetic damage from direct exposure to commercial samples of 2,4-D should not be ignored.

145. COMPARATIVE METABOLISM AND TOXICITY OF CHEMICAL CARCINOGENS IN PRIMARY CULTURES OF HEPATOCYTES

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In Vitro Cell Dev Biol; 21(9):485-94 1985

Hepatocytes were prepared by an *in situ* or biopsy perfusion of liver with collagenase. Hepatocytes from adult livers were cultured without serum on collagen-coated dishes in culture medium supplemented with hormones. Stable monolayers were established within 24 h and were maintained for up to 10 d. The hormone supplement maintained cytochrome P-450, a critical component of mixed function oxygenase responsible for activation of many procarcinogens. The addition of serum and phenobarbital to the cultures also maintained higher levels of mixed function oxygenase activity. Viable cultures were prepared from mice, rats, guinea pigs, rabbits, dogs, monkeys, and humans. Metabolism studies revealed the rate of metabolism and the extent of covalent binding to macromolecules, including DNA. Measures of cytotoxicity and genotoxicity *in vitro* provide an indication of hepatocarcinogenic and hepatocarcinogenic potency *in vivo*. Comparative metabolism, cytotoxicity, and genotoxicity studies provide a means of facilitating the extrapolation of toxicity data from laboratory animals to humans.

146. THE GENOTOXICITY OF AROMATIC AMINES IN PRIMARY HEPATOCYTES ISOLATED FROM C57BL/6 AND DBA/2 MICE

Miller ME, Glavinski JB, Thorgeirsson SS

Laboratory of Experimental Carcinogenesis, National Cancer Institute, Bethesda, MD

Carcinogenesis; 5(5):757-804 1984

The capacity of the chemical carcinogen 2-acetylaminofluorene (AAF) and its derivatives to cause DNA damage in primary mouse hepatocytes from aryl-hydrocarbon responsive C57BL/6 and non-responsive DBA/2 mice was studied using the alkaline elution technique. Low levels of DNA damage were observed after exposure of hepatocytes to either AAF or 2-aminofluorene (AF) (50-100 μ M). Quantitation of meta bolites produced from AAF in hepatocytes from untreated C57BL/6 and DBA/2 mice using h.p.l.c. showed a similar metabolic profile with respect to C- and N-hydroxylations. After *in vivo* pretreatment with the potent monooxygenase inducer TCDD (50 micrograms/kg), N-hydroxylation in the C57BL/6- and DBA/2-derived hepatocytes increased 25- and 3-fold, respectively. However, the C-hydroxylation pathways were still responsible for approximately 90% of the metabolism in cells from both strains. This may explain why only a slight increase in the DNA damage was observed in C57BL/6 mouse hepatocytes after incubation with AF or AAF and no increase in DNA damage was seen in the DBA/2 hepatocytes isolated from TCDD treated animals. Both N-hydroxy-2-acetylaminofluorene (N-OH-AAF) and N-acetoxy-2-acetylaminofluorene (N-OAc-AAF) caused clear dose-dependent increases in DNA strand breaks (5-100 μ M), suggesting that N-hydroxylation was the rate limiting step in the activation process of AAF leading to the DNA damage. Treatment of hepatocytes with paraquat, an inhibitor of microsomal deacetylase activity, prior to exposure to either N-OH-AAF or N-OAc-AAF completely in-

hibited the damage caused by N-OH-AAF, while the damage caused by N-OAc-AAF was only partially inhibited. This suggests that these compounds are causing genotoxic effects after deacetylation. In accordance with this, N-hydroxy-2-aminofluorene (N-OH-AF), the deacetylated metabolite of N-OH-AAF, was an effective genotoxic agent, causing DNA strand breaks at low doses. Depletion of cellular glutathione by pretreatment with diethyl maleate, increased the sensitivity of the cells to the damage induced by N-OH-AF. These data indicate that glutathione may play an important role in the detoxification of N-OH-AF in mouse hepatocytes.

147. EFFECT OF HERBICIDE 2,4,5-TRICHLOROPHENOXYETHANOL (TCPE) CONTAINING DIOXIN ON MUTATION AND INDUCTION OF SISTER CHROMATID EXCHANGES

Tóth K, Gáth E, Somlai-Réti S, Szegő J

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Carcinogenesis; 5(12):1725-8 1984

Previous studies have indicated that both herbicide 2,4,5-trichlorophenoxyethanol (TCPE) and its contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) enhance liver tumor incidence in male Swiss mice in a dose-dependent manner. In this report the mutagenicity of TCPE (containing 0.1 p.p.m. TCDD) in the *Salmonella/microsome* assay was studied, and the effect of this herbicide on the colony-forming ability and on the frequency of sister chromatid exchange (SCE) in Chinese hamster cells were determined. TCPE (1-500 micrograms/plate) appeared non-mutagenic in strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 either in the absence or presence of S-9 mix from male Wistar rat, male Swiss mouse and male hamster liver pre-treated by Aroclor 1254, using the standard plate incorporation and the pre-incubation assay. Male mouse urine, after a single administration of TCPE to the animal, also proved to be non-mutagenic in strains TA 98 and TA 100. In mammalian cell systems, however, TCPE directly induced a dose related increase in SCE frequency. The dose required to double the control SCE frequency was in the slightly toxic range of the survival curve for TCPE. The cytotoxicity was diminished both in bacterial and mammalian systems in the presence of S-9 mix from male Swiss mouse liver. This S-9 mix strongly reduced SCE frequency induced by TCPE. The induction of SCE observed cannot be attributed to the contaminant TCDD alone, because the SCE production was not influenced by the appropriate low concentration of pure TCDD.

148. PESTICIDE INDUCED DNA DAMAGE AND ITS REPAIR IN CULTURED HUMAN CELLS

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Dept. Biophysics, Ohio State Univ., Columbus, OH 43210

Mutat Res; 42(2): 161-174 1977

The effects of 13 pesticides (lindane, rotenone, chlordane, heptachlor, heptachlor-epoxy, DDT, aldrin, dieldrin, carbaryl, diaquat, 2,4-D fluid, dimethoate, captan, at concentrations of 1, 10, 100, and 1,000 μ M), on the induction of unscheduled DNA synthesis in simian virus 40 (SV40)-transformed human cells (VA-4) in culture, with and without metabolic activation by rat liver microsomes were studied. All of the agents except lindane, rotenone, and DDT, either directly or upon metabolic activation, induced unscheduled DNA synthesis. The DNA repair kinetics and the size of the repaired regions resulting from treatment with four agents (carbaryl, chlordane, dieldrin, and 2,4-D fluid) were studied by 313-nanometer photolysis of repaired regions containing bromodeoxyuridine. The size of the repaired regions differed among the four, but the type of repair could be classi-

fied as either the x-ray (short) or UV-ray (long) type. (63 Refs)

149. ENVIRONMENTAL GENETICS: A MODEL TO INVESTIGATE POLLUTANTS PRODUCING GENETIC DAMAGE.

Arnold FM

Ohio State Univ., Columbus, OH 43210

Dis. Abstr. Int. Biol. 36/81:3737 1976

New and simplified ways to screen genetic damage-producing environmental pollutants were developed. Thirteen compounds (Lindane; Rotenone; Chlordane; Heptachlor; Heptachlor-epoxy; Aldrin; Dieldrin; Carbaryl; Diquat; 2,4-D fluid; Dimethoate; and Captan) were used to study unscheduled DNA synthesis by autoradiography. This study showed that some compounds acted as ultimate carcinogens that did not require any metabolic activation, that some compounds were precarcinogens requiring activation (with liver microsomal fraction and cofactors nicotinamide adenine dinucleotide (NAD), NAD phosphate and glucose-6-phosphate; Carbaryl was given *Azobacter vinelandii*) and that some compounds did not induce unscheduled DNA synthesis (excluded from further study). Human fibroblasts VA-4 (transformed with simian virus 40) were used to study the kinetics of DNA damage repair by bromodeoxyuridine photolysis after exposure to the compounds studied. Saturation photolysis was seen after 13 min exposure to 313 nm radiation; it was concluded that there was a decrease in molecular weight of the resulting DNA fragments. Mutation frequency was studied using Chinese hamster cells V-79 (spontaneously transformed). The sensitivity of the plasma membrane to ouabain was used as a genetic marker. In all cases, the mutation rate was increased. It was concluded that the effectiveness of demonstrating DNA repair and measuring the mutation rate to screen for genetically dangerous pollutants was confirmed.

150. THE BACTERIAL MUTAGENICITY OF NITROPOLYCHLORINATED DIBENZO-P-DIOXINS

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College Station 77843

Mutat Res. 16/91-21:17-22 1986

The bacterial mutagenicity of 2-nitrodibenzo-p-dioxin, a mixture of 2-nitro-7-chloro- and 2-nitro-8-chlorodibenzo-p-dioxin, 7-nitro-2,3-dichloro-, 8-nitro-2,3,7-trichloro-, 2-nitro-1,3,7,8-tetrachloro- and 3-nitro-1,2,4,7,8-pentachlorodibenzo-p-dioxin was determined using *Salmonella typhimurium* tester strains TA98 and TA100 with and without rat hepatic S9 for metabolic activation. All the nitro-PCDDs exhibited some direct-acting mutagenicity with both tester strains, however, the activity was significantly lowered in the presence of exogenous S9 and the compounds were more mutagenic to tester strain TA98. The mutagenicity of the nitro-PCDDs was also dependent on structure because there was a marked decrease in activity with increasing chlorine content. Because nitro-PCDDs have recently been identified as incomplete combustion products of municipal waste, this study confirms that this new class of compounds contains some bacterial mutagens.

151. GENOTOXICITY OF HETEROCYCLIC AMINES IN THE SALMONELLA/HEPATOCYTE SYSTEM

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Institute, Bethesda, Maryland 20892

Jpn J. Cancer Res. 76/91:833-43 1985

The *Salmonella*/hepatocyte system was employed to determine the mutagenicity in bacteria as well as the DNA damage induced in mouse hepatocytes following exposure to heterocyclic amines. With hepatocytes from C57BL/6N mice,

3-amino-1-methyl-5H-pyrido(4,3-b)indole (Trp-P-2) and 2-amino-3-methylimidazo(4,5-f)quinoline (IQ) showed a clear mutagenic effect in the *Salmonella*, while weak mutagenic effects were observed with 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indole (Trp-P-1), 2-amino-6-methyldipyrido(1,2-a:3',2'-b)imidazole (Glu-P-1), and 2-aminodipyrido(1,2-a:3',2'-d)imidazole (Glu-P-2). All the compounds induced low levels of DNA damage in the hepatocytes. In vivo pretreatment of mice with the potent monooxygenase inducer 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 50 micrograms/kg) clearly increased both the mutagenicity in the bacteria and the DNA damage induced in the hepatocytes in vitro. Glu-P-2 showed the lowest mutagenic effect but induced more DNA damage at low concentrations than the other compounds when TCDD-pretreated hepatocytes were used. These data indicate that the genotoxic potency of Glu-P-2 in the intact hepatocyte differs from that observed in the bacteria. Treatment of hepatocytes with α -naphthoflavone, a selective inhibitor of polycyclic hydrocarbon-inducible cytochrome P-450 form(s), prior to exposure to the heterocyclic amines completely inhibited the mutagenic effect in the bacteria. In vivo administration of all the heterocyclic amines 4 hr prior to isolating the hepatocytes resulted in DNA damage, and this effect was augmented by TCDD pretreatment of mice. Our data suggest that agents modulating the activity and composition of the cytochrome P-450 system may greatly influence both toxicity and carcinogenicity of these heterocyclic amines.

152. MUTAGENICITY TESTING OF AGENT ORANGE COMPONENTS AND RELATED CHEMICALS

Martelians K, Haverth S, Speck W, Zenger E

Microbial Genetics Department, SRI International, Menlo Park, California

Toxicol Appl Pharmacol. 75/11:137-46 1984

Components of the herbicide Agent Orange - 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and their esters, and the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) - and related chemicals were tested for mutagenicity using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. No mutagenic activity was observed for any of the chemicals tested.

153. MUTAGENICITY OF LICHEN CONSTITUENTS

Shikama T, Wei C

Department of Environmental Toxicology, University of California,

Davis

Environ Mutagen. 6/51:737-62 1984

Usnic acid (the most abundant lichen constituents), physodic, and physodalic acids isolated from *Hypogymnia enteromorpha* (Ach.) Nyl. were tested for mutagenicity in the Ames *Salmonella*/microsome assay. Physodalic acid exhibited clear dose-related mutagenicity against *Salmonella typhimurium* strain TA 100 with or without S9 mix in both plate-incorporation and preincubation assays. The addition of S9 mix increased the number of revertants approximately threefold and fourfold in preincubation and plate-incorporation assays, respectively.

154. MUTAGENICITY AND DNA DAMAGE INDUCED BY ARLAMINES IN THE SALMONELLA/HEPATOCYTE SYSTEM

Suzano N, Erickson L, Smith CL, Morrison E, Thorgeirsson SS

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Carcinogenesis. 4/21:161-167 1983

Coincubation of isolated, intact rat hepatocytes with *Salmonella typhimurium* tester strain TA 98 (*Salmonella*/hepatocyte

cyte system) has been employed to determine both bacterial mutagenicity and DNA damage in rat hepatocytes following treatment with 2-acetylaminofluorene (AAF) and its derivatives. In vivo pretreatment of rats with either 2,3,7,8-tetrachlorodibenzo-dioxin or 3-methylcholanthrene markedly increased both DNA damage and bacterial mutation frequency upon incubation of AAF or 2-aminofluorene (AF) in this system. The increase in damage to the hepatocyte DNA was more pronounced after AAF treatment than following AF exposure, while the increase in bacterial mutation frequency was greater after AF treatment. Treatment of hepatocytes with paracetamol prior to exposure to N-hydroxy-2-acetylaminofluorene (N-OH-AAF) or N-acetoxy-2-acetylaminofluorene (N-OAc-AAF) partially inhibited both DNA damage and the bacterial mutagenicity caused by these agents. Treatment of primary rat hepatocytes with 2-hydroxy-2-aminofluorene (N-OH-AF) causes a low level of DNA breaks. Substitution of primary rat hepatocytes with highly differentiated rat hepatoma cells (Reuber H4-II-E) revealed a low level of DNA breakage after exposure to N-OH-AAF whereas treatment with either N-OAc-AAF or N-OH-AF induced a dose dependent increase in DNA breaks. Pretreatment of the Reuber cells with paracetamol inhibited the DNA damage caused by N-OAc-AAF whereas the DNA damage induced by N-OH-AF was increased after paracetamol treatment. Employing host cells with differing metabolic capacity, such as Reuber vs primary hepatocytes, in the Salmonella/hepatocyte system, may allow a determination of the relative importance of different metabolic pathways in mutagenicity and/or genotoxicity of arylamines. (Author abstract) (23 Refs)

155. MUTAGENESIS OF NITRO-DIBENZO-P-DIOXINS

White WE Jr, Rock SG
Department of Biochemistry, University of Alabama in Birmingham.
Drug Chem Toxicol; 6(1):71-82 1983

Two dioxins, 1-nitro-dibenzo-p-dioxin (I) and 2,3-dichloro-7-nitro-dibenzo-p-dioxin (II), were prepared by condensing 3,4-dichloro nitrobenzene with the dipotassium salts of catechol and 4,5-dichloro-catechol, respectively. Both compounds were very mutagenic for *Salmonella typhimurium* TA1538, but only weakly mutagenic for TA1537. Compound II, but not compound I, also induced a few (approximately 100 revertants at near toxic doses) point mutations in strain TA1535.

156. MUTAGENICITY ASSAYS WITH 2,4-DICHLOROPHENOXYACETIC ACID - AMINO ACID CONJUGATES

Rock SA, Maxam RO
J Agric Food Chem; 31(6):1571-2 1983

Keywords (MeSH): *Amino Acids; Comparative Study; Mutagenicity Tests; *Mutagens; *Salmonella Typhimurium*/drug effects/genetics; Species Specificity; Structure-Activity Relationship; 2,4-Dichlorophenoxyacetic Acid/*analogues and derivatives/toxicity.

157. MUTAGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND PERFLUORO-N-DECANOIC ACID IN L5178Y MOUSE-LYMPHOMA CELLS

Rogers AM, Andrus ME, Bach KC
Microbiological Associates, 3221 River Road, Bethesda, MD, 20816
Mutat Res; 10(1):445-449 1982

The toxicity and mutagenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and perfluoro-n-decanoic acid (PFDA) in L5178Y mouse-lymphoma cells were investigated. Treatment of cells with PFDA had no effect on suspension growth below 50 ug/ml, and doses above 100 ug/ml caused cell lysis. No effect on suspension growth was noted for TCDD in doses up to 0.50 ug/ml. In soft agar, TCDD at greater

than 0.01 ug/ml caused a change in clone formation and reduced plating efficiency. PFDA at 0.5 ug/ml for 24 hr markedly reduced plating efficiency without affecting suspension growth. TCDD significantly induced mutation in the methylenes, excess thymidine, and thioguanine selective systems. No significant mutation was noted in the ouabain or cytosine arabinoside selective systems. Treatment of L5178Y cells with PFDA resulted in no significant mutation induction in any of the five selective systems. In L5178Y cells, TCDD appeared to be as mutagenic as proflavin, while PFDA was not mutagenic in these cells. (13 Refs)

158. MUTAGENICITY TESTING OF CHLORINATED BIPHENYLS AND CHLORINATED DIBENZOFURANS

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Mutat Res; 10(1):45-56 1982

The *Salmonella typhimurium*/microsome assay of Ames was used to assay four chlorinated biphenyls and five chlorinated dibenzofuran compounds. None of the polychlorinated biphenyls (PCB: 4-chlorobiphenyl, 3,4,3',4'-tetrachlorobiphenyl, 2,4,2',4'-tetrachlorobiphenyl, and 2,4,6,2',4',6'-hexachlorobiphenyl) reverted any of the *Salmonella* tester strains, which included three DNA target sequences. Assays incorporating hepatic extracts (S9) from rats treated with various inducing agents were also negative. Thus, S9 preparations from PCB-treated rats were not able to activate PCB components to detectably mutagenic forms in vitro. Dibenzofuran was assayed using dimethylsulfoxide as a solvent; the chlorinated derivatives were dissolved in a mixture of benzene:isooctane (1:9). Dibenzofuran, 2,9-dichlorodibenzofuran, 3,6-dichlorodibenzofuran, 2,3,7,8-tetrachlorodibenzofuran, and octachlorodibenzofuran were nonmutagenic for *Salmonella* strains TA98 and TA100 over a 3-log dose range with or without S9 activation. (37 Refs)

159. GENETIC TOXICOLOGY STUDIES WITH 2-NITROBENZOFURANS AND 2-NITRONAPHTHOFURANS

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Mutat Res; 10(1):31-40 1982

The mutagenic activity of three 2-nitronaphtho[2,1-b]furans, two 2-nitronaphtho[3,2-b]furans, and two 2-nitronaphtho[1,2-b]furans was tested using the *Salmonella*/microsome assay (Mutatest). The seven compounds gave a mutagenic response with the following characteristics: it occurs on strains TA1537, TA1538, TA98, and TA100, but not on strain TA1535; the mutagenic response is partially dependent on the bacterial nitroreductase activity and is lower in the presence of an activating mixture S9 from rat liver; it varies quantitatively and qualitatively with the structure of the compound. The mutagenic responses of the 2-nitronaphthofurans and three 2-nitrobenzofurans was also examined in a phase-induction assay (Inductest). The mutagenic potency measured in the Mutatest correlated well with that measured by the Inductest; those compounds which were negative in the Mutatest were also negative in the Inductest. The mutagenic activity of the two most potent mutagens examined was tested using strains TA1978 and TA2421, the UVr+ counterparts of the TA1538 and TA98 uvrB excision-repair mutants used in the Mutatest. The mutagenic response was reduced by at least a factor of 100 compared to the uvrB mutants, indicating that the excision-repair system is efficient in preventing the mutagenic effect of the 2-nitronaphthofurans. 2-

Nitro-7-methoxynaphtho[2,1-b]furan (R7000), one of the most potent compounds, also induced sperm abnormality in mice (Spermanest). The results of the bacterial tests and Spermanest in relation to the possible genetic toxicity of these compounds is discussed. (11 Refs)

140. MUTAGENICITY TESTING OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN HISTIDINE AUXOTROPHS OF SALMONELLA TYPHIMURUM

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Toxicol Appl Pharmacol: 59(1):125-129 1981

The mutagenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was investigated with *Salmonella typhimurium* histidine auxotrophs TA1535, TA100, TA1538, and TA1537. No mutagenicity was found in any of these strains in the presence of mammalian metabolic activating systems isolated from the livers of rats and hamsters treated with Aroclor 1254 or hamsters treated with TCDD. No mutagenicity was observed in studies without NADP+-dependent metabolic activation. (25 Refs)

141. MUTAGENIC ACTIVITY OF BENZOFURANS AND NAPHTHOFURANS IN THE SALMONELLA/MICROSOME ASSAY: 2-NITRO-7-METHOXY-NAPHTHO[2,1-B]FURAN (R7000), A NEW HIGHLY POTENT MUTAGENIC AGENT

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Mutat Res: 80(4):555-562 1981

Mutagenic properties of a series of 14 2-nitrobenzofurans (NBFs), 2-nitronaphthofurans (NNFs), and furans devoid of a nitro group were studied. Mutagenicity was evaluated by means of a standard plate assay utilizing *Salmonella typhimurium* tester strains (TA1535, TA1537, TA1538, TA98, TA100, and TA98NR) in the presence or absence of a liver microsome activating mixture (S9). No mutagenic activity was detected for compounds devoid of a nitro group at position 2 of the heterocycle, with one exception. The exception, 2-acetyl-7-methoxynaphtho[2,1-b]furan, gave an extremely weak mutagenic response in comparison to that of the other NNFs. Mutagenic potency of the active compounds was reduced when a tester strain deficient in nitroreductase activity (TA98NR) was used. Mutagenic activity in the remaining strains was higher in the absence of the S9 mixture than in its presence; however, none of the compounds tested was mutagenic in strain TA1535 in the presence or absence of the S9 mixture. The mutagenic potency of the active compounds varied considerably depending on various transformations that can affect the nitroheterocycle. For the NBFs, the activity was approx the same on strains TA1537 and TA1538, 5-10x higher on strain TA98, and 100x higher on strain TA100. For the NNFs, the mutagenic potency was approx 2 orders of magnitude higher than for the NBFs. The activity of the NNFs was lower on TA98 than on TA1537 and TA1538 and was approx 5x higher for TA100 than for TA1537 and TA1538. One of the tested compounds, 2-nitro-7-methoxynaphtho[2,1-b]furan, showed a particularly high mutagenic potency: in tester strain TA100, this compound produced about 200,000 revertants/nanomole of compound. (25 Refs)

142. SEARCHING FOR GENETIC EFFECTS OF TOXIC CHEMICALS: CHROMOSOMAL ABERRATION AND SISTER CHROMATID EXCHANGE (4 Refs)

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Bull Environ Contam Toxicol: 33(6):710-23 1984

Keywords (MeSH): Animal; Cell Nucleus/drug effects/pathology; *Chromosome Aberrations; Chromosome Abnormalities/chemically induced/*familial and genetic; Chromosome Banding; Chromosome Deletion; Dioxins/*adverse effects; Human; Lymphocytes/drug effects/pathology; Mice; Neoplasms/chemically induced/familial and genetic; Review; Sister Chromatid Exchange/*drug effects.

B.

PROMOTION OF CELL TRANSFORMATION

143. EFFECT OF THE TUMOR PROMOTERS 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) UPON INTERCELLULAR COMMUNICATION BETWEEN C3H/10T1/2 CELLS (MEETING ABSTRACT)

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Proc Annu Meet Am Assoc Cancer Res: 27:133 1986

Sustained inhibition of intercellular communication (IC) has been suggested as the mechanistic basis for the promotion of cell transformation. The effects of the tumor promoters TPA and TCDD upon IC between nontransformed C3H/10T1/2 cells were determined by microinjection of cells with Lucifer Yellow dye throughout the course of 43 day cell transformation experiments. IC in the absence of promoters varied as a function of growth state. Injection of cells in proliferating cultures (up to day 10) produced networks of 6-20 fluorescent recipient cells. IC decreased as the cultures approached confluence, but resumed (up to 60 cell networks) upon the establishment of smooth confluent monolayers (days 15-43). Treatment with 250 ng/ml TPA from day 5 to 43 produced a transient (less than 24 hr) inhibition of both IC and growth on day 5; a sustained inhibition of IC on days 10-19 as the cultures attained confluence; but had no effect upon IC after confluent monolayers were established (days 20-43). Treatment with 100 pM TCDD from day 5 to 43 promoted morphological transformation, but did not inhibit IC or affect growth at any stage of the assay. In contrast, little IC occurred between cells in foci produced by TPA or TCDD, or between focus and monolayer cells. A simple generalized inhibition of IC in nontransformed C3H/10T1/2 cells does not appear to be necessary for promotion by TCDD and may be necessary, but not sufficient, for promotion by TPA. However, alterations in IC which specifically influence transformed or 'initiated' cells could be an important determinant in focus development.

144. EFFECT OF POLYCHLORINATED DIBENZO-P-DIOXINS UPON C3H/10T1/2 CELL TRANSFORMATION (MEETING ABSTRACT)

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Proc Annu Meet Am Assoc Cancer Res: 26:130 1985

Some polychlorinated dibenzo-p-dioxins are potent rodent carcinogens. Assessment of the carcinogenic risk these environmental pollutants pose for man has been complicated by the diversity of isomeric dioxin analogs present in the environment, the wide range of carcinogenic potencies exhibited

by these analogs, and limited understanding of the mechanism(s) of dioxin carcinogenicity. Studies were undertaken to evaluate use of the C3H/10T1/2 cell transformation system for the study of carcinogenic dioxins. 2,7-Dichloro (DCDD), 2,3,7,8-tetrachloro (TCDD), and 1,2,3,4,7,8-hexachloro (HCDD) dibenzo-p-dioxin were nonsystemic up to the limits of aqueous solubility (1-10 μ M), failed to transform C3H/10T1/2 cells, and did not initiate transformation in cultures subsequently exposed to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate. However, TCDD was a promoter of transformation when continuously applied to cultures initiated with 3.7 μ M N-methyl-N'-nitro-N-nitrosoguanidine. Responses were observed at TCDD concentrations as low as 4 pM; higher concentrations produced up to 9-fold enhancement of focus formation. HCDD produced promotion responses comparable in magnitude to those of TCDD but required higher (12 pM) concentrations to elicit an effect. Five μ M DCDD was required to produce twofold enhancement of transformation. The ability to promote transformation thus correlates with the probable carcinogenic potencies of these compounds. Furthermore, while promotion of transformation by phorbol esters is extremely serum lot dependent, 7 of 8 fetal bovine serum lots tested were suitable for dioxin promotion experiments. These studies suggest dioxin carcinogenicity may be mediated by tumor promoting activity and that the C3H/10T1/2 cell transformation system is a relevant *in vitro* model for their study.

145. 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) PROMOTES THE TRANSFORMATION OF C3H/10T1/2 CELLS

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Carcinogenesis, 64(1):531-3 1983

Continuous treatment of C3H/10T1/2 cells with low concentrations (greater than or equal to 4 pM) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) enhanced focus production in cultures pretreated with N-methyl-N'-nitro-N-nitrosoguanidine. Maximal enhancement occurred at 40 pM TCDD, a concentration 10 000-fold lower than that required to produce an optimal response with 12-O-tetradecanoylphorbol-13-acetate. Single treatments with 0.06 nM-5 μ M TCDD did not transform C3H/10T1/2 cells or initiate the process of transformation in cultures subsequently exposed to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate. Promotion of transformation is thus the predominant effect of TCDD in the C3H/10T1/2 cell transformation system.

146. INITIATION AND PROMOTION IN CULTURES OF C3H/10T1/2 MOUSE EMBRYO FIBROBLASTS (MEETING ABSTRACT)

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Symposium on Tumor Promotion and Enhancement in the Etiology of
Human and Experimental Respiratory Tract Carcinogenesis, June
18-20, 1984, Williamsburg, VA, U.S. Environmental Protection
Agency, p. 36, 1984.

The transformation of C3H/10T1/2 mouse embryo fibroblasts can proceed through discrete stages of initiation and promotion. Initiation of transformation is the principal effect of many chemical carcinogens upon asynchronous C3H/10T1/2 cells. Treatment with these agents will not produce transformed foci unless treated cultures are subsequently exposed to a tumor promoter such as 12-O-tetradecanoylphorbol-13-acetate (TPA). Several aspects of initiation and promotion in C3H/10T1/2 cells parallel observations made in mouse skin systems. Treatment with either an initiating agent

or a tumor promoter alone has little effect. However, a single treatment with an initiating agent followed by multiple tumor promoter applications will produce a large number of transformed foci. Enhancement of focus production will not occur if the tumor promoter is applied before the initiating agent. Dose responses for the promotion of transformation are consistent with the existence of thresholds and dose response plateaus. Finally, just as many mouse skin papillomas will regress if TPA treatment is discontinued, termination of TPA treatment in cell culture results in the 'regression' of many transformed foci. The specificity of the C3H/10T1/2 system is such that responses are obtained with promoters such as TPA, mezerein, formaldehyde, and TCDD. Negative responses are obtained with compounds such as phorbol and 4-O-methyl-TPA. This response profile indicates C3H/10T1/2 cells are useful for the detection and study of mouse skin tumor promoters and suggest the system may be specific for chemicals with Stage II promoting activity.

147. EFFECT OF PROMOTING AND NON-PROMOTING CHEMICALS UPON INTERCELLULAR COMMUNICATION BETWEEN C3H/10T1/2 CELLS (MEETING ABSTRACT)

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Proc. Ninth Meet. Am. Assoc. Cancer Res., 23:143 1984

Inhibition of intercellular communication (IC) has been proposed as a mechanism for the promotion of carcinogenesis by chemicals. We have observed that the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibits IC between C3H/10T1/2 cells, but that the effect is transient and occurs at TPA concentrations far lower than those required to promote transformation (Dorman et al, Carcinogenesis 3:1107, 1983). In the present study the ability of several chemicals to inhibit IC was studied by autoradiographic analysis of their effect upon 3H-uridine transfer from 3000 prelabelled C3H/10T1/2 cells to 300,000 unlabelled transformed C3H/10T1/2 cells. These results were compared to the chemical's ability to promote transformation in cultures initiated with 0.5 μ g/ml N-methyl-N'-nitro-N-nitrosoguanidine. Mezerein (0.25-2500 ng/ml) inhibited IC in a concentration dependent manner from 1.5 to 80.6%. Preexposure to 250 ng/ml mezerein for 12-72 hr prevented inhibition of IC by a second exposure. Promotion of transformation by mezerein (8-fold enhancement of focus formation) required concentrations of 100 ng/ml or higher. 4-O-methyl-TPA (0.25-250 ng/ml) did not inhibit IC or promote transformation. 2.5 μ g/ml 4-O-methyl-TPA inhibited IC by 78%, but was also cytotoxic and did not promote transformation. The nonpromoting solvent dimethylsulfoxide (0.25-2.0%) failed to inhibit IC. Treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 6 pM-6 nM), a promoter of C3H/10T1/2 transformation at concentrations as low as 4 pM, did not inhibit IC. Thus, while a transient inhibition of IC in the absence of cytotoxicity appears to correlate with the promoting ability of some chemicals in C3H/10T1/2 cultures, it does not appear to be necessary for promotion by TCDD. (1 Ref)

C. IN VITRO TOXICITY MODELS

148. TOXICITY OF CHLORINATED AROMATIC COMPOUNDS IN ANIMALS AND HUMANS: IN VITRO APPROACHES TO TOXIC MECHANISMS AND RISK ASSESSMENT

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Environ Health Perspect; 60:69-76 1983

Human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and chlorinated analogs commonly results in pathological changes in the skin and its appendages characterized by thickening of the epidermis (acanthosis), hyperkeratosis and squamous metaplasia of the epithelial lining of the sebaceous glands. Acneiform lesions (chloracne) develop as hair follicles dilate and fill with keratin and sebaceous glands become cystic. In animal models it has been found that the chloracnogenic potential of the halogenated aromatic compounds examined corresponds with the relative affinity of these same compounds for the cytosolic TCDD receptor. This receptor controls the coordinate expression of a number of inducible enzyme activities and in certain cell targets can alter normal programs of proliferation and differentiation. In this report we describe some of our ongoing studies on the mechanisms of action of TCDD in normal human epidermal cells and squamous cell carcinoma (SCC) lines. These systems permit detailed investigation of the molecular and biochemical events underlying pathologic changes in the skin and offer the potential of establishing a risk assessment model for halogenated aromatic compounds by using human target cells. (41 Refs)

149. STRAIN DIFFERENCES IN SENSITIVITY OF NORMAL HUMAN EPIDERMAL CELLS TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) (MEETING ABSTRACT)

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Chem. Ind. Ind. Toxicol. Res. Tri. Park, NC 27709
Fed Proc; 44(6):1842 1983

We have reported that TCDD stimulates terminal differentiation in cultures of normal human epidermal cells, analogous to epidermal hyperkeratosis observed in vivo. To determine whether these cells are an appropriate model to assess interindividual differences in sensitivity to TCDD, the concentration-dependence for the TCDD-induced modulation of epidermal growth factor (EGF) binding and 7-ethoxycoumarin O-deethylase (ECOD) activity was quantitated in 4 human epidermal cell strains derived from different individuals. In 3 strains treated with TCDD, the EC50 value for both the decrease in EGF binding and the increase in ECOD activity was 1 nM, whereas in the 4th strain the EC50 value for modulation of both responses by TCDD was 0.1 nM. Regulation of EGF binding by TCDD appeared to occur predominantly in the basal cell population. Differential sensitivity to TCDD for induction of ECOD activity, a marker for Ah receptor-mediated responses, is analogous to murine strain differences in sensitivity regulated by the Ah locus [BBRC 115:611 (1983)]. Modulation of EGF binding by TCDD is linked to hyperkeratinization [Osborne and Greenlee, 1983, TAP, in press]. Thus, cultured human epidermal cells appear to be a suitable in vitro system to study mechanisms regulating genetically-determined differences in sensitivity of biochemical responses relevant to TCDD toxicity. (2 Refs)

170. ACUTE EFFECTS OF 12-O-

TETRADECANOYLPHORBOL-13-ACETATE, TELEOCIDIN B, OR 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON CULTURED NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS

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Carcinogenesis; 5(2):209-15 1984

Effects of teleocidin B, 12-O-tetradecanoylphorbol-13-acetate (TPA), phorbol, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,7-dichlorodibenzo-p-dioxin (DCDD) on normal human bronchial epithelial cell cultures were assessed by quantitation of cellular morphology, clonal growth (population doublings per day), cross-linked envelope (CLE) formation and the enzymatic activities of aryl hydrocarbon hydroxylase (AHH), ornithine decarboxylase (ODC) and plasminogen activator (PA). Toxicity was assessed by clonal growth assays. Teleocidin B and TPA had similar effects on growth, morphology and enzyme activities. When the cells were incubated with TPA or teleocidin B at concentrations of 1-100 nM for 6 h, RNA synthesis was unaffected, but DNA synthesis decreased and squamous differentiation, marked by an increase in cell surface area and cross-linked envelope formation, was increased. TPA and teleocidin B also increased ODC activity in LHC-0 medium (a maintenance medium without epidermal growth factor) but caused a decrease of ODC activity in LHC-4 (a growth medium containing epidermal growth factor). Finally, TPA and teleocidin B each caused an increase of PA and a decrease of AHH activities in both media. Phorbol, a non-promoting analogue of TPA, had no effect on growth, morphology or biochemical assays. TCDD (100 nM) caused a 15% decrease in cell growth when cells were incubated in LHC-4, and this was accompanied by an increase in cell surface area, PA activity, and CLE formation. TCDD caused an increase in AHH and ODC activities when the cells were incubated in either LHC-0 or LHC-4 medium. DCDD did not alter cell growth, and its morphological and biochemical effects were similar to those of TCDD although less marked. In conclusion, results reported here are consistent with the hypothesis that an important property of some tumor promoters is their ability to induce terminal differentiation in normal, non-initiated epithelial cells.

171. 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN INDUCES HYPERPLASIA IN CONFLUENT CULTURES OF HUMAN KERATINOCYTES

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J Invest Dermatol; 82(5):532-4 1984

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the prototype for a group of halogenated aromatic hydrocarbons which can be potent modulators of growth and differentiation of epithelial tissues. TCDD causes chloracne and can act as a skin tumor promoter, but these actions have been demonstrated only in animals in which TCDD causes epidermal hyperplasia. Study of the hyperplastic response to TCDD has been hampered by lack of an in vitro model; all previous investigations indicated that TCDD had no in vitro effect on cell growth. We show here that nanomolar concentrations of TCDD cause hyperplasia in confluent cultures of human keratinocytes and suggest that this model system will be useful for analyzing mechanisms of TCDD-induced epithelial hyperplasia and genetic differences in responsiveness to TCDD.

172. AN IN VITRO APPROACH TO STUDYING CUTANEOUS METABOLISM AND DISPOSITION OF TOPICALLY APPLIED XENOBIOTICS

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Toxicol Appl Pharmacol: 75(2):295-300 1984

This extent to which cutaneous metabolism may be involved in the penetration and fate of topically applied xenobiotics was examined by metabolically viable and structurally intact mouse skin in organ culture. Evidence that skin penetration of certain chemicals is coupled to cutaneous metabolism was based upon observations utilizing [^{14} C]benzo[a]pyrene (BP). As judged by the recovery of radioactivity in the culture medium 24 hr after in vitro topical application of [^{14} C]BP to the skin from both control and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced C3H mice, skin penetration of BP was higher in the induced tissue. All classes of metabolites of BP were found in the culture medium; water-soluble metabolites predominated and negligible amounts of unmetabolized BP were found. As shown by enzymatic hydrolysis of the medium, TCDD induction resulted in shifting the cutaneous metabolism of BP toward the synthesis of more water-soluble conjugates. Differences in the degree of covalent binding of BP, via diol epoxide intermediates to epidermal DNA, from control and induced tissues were observed. These differences may reflect a change in the pathways of metabolism as a consequence of TCDD induction. These results indicated that topically applied BP is metabolized by the skin during its passage through the skin; and the degree of percutaneous penetration and disposition of BP was dependent upon the metabolic status of the tissue. This suggests that cutaneous metabolism may play an important role in the translocation and subsequent physiological disposition of topically applied BP.

173. CYTOTOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON PRIMARY CULTURES OF ADULT RAT HEPATOCYTES

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Arch Environ Contam Toxicol: 12(2):183-188 1983

In cultured liver cells from adult male Sprague-Dawley rats, one po dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD: 5-25 μ g/kg) 2-30 days previously, caused a dose-dependent depression of ouabain uptake at all study intervals. Dexamethasone-induced tyrosine aminotransferase activity and alpha-aminobutyrate uptake were also depressed in hepatocyte cultures from rats given 10-25 μ g/kg of TCDD, but not at the lowest dose. TCDD was not directly toxic to hepatocyte cultures. These effects were not observed in hepatocytes from rats treated with 1,3,6,8-tetrachlorodibenzo-p-dioxin. (28 Refs)

174. KERATINIZATION OF MOUSE TERATOMA CELL LINE XB PRODUCED BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN: AN IN VITRO MODEL OF TOXICITY

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Cell Carcin: 22(1):27-36 1980

An extremely potent toxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), serves as the prototype of a large group of halogenated aromatic hydrocarbons, all of which produce a similar and characteristic pattern of toxic lesions, induce a battery of coordinately expressed enzymes, and appear to exert both their toxic effects and enzyme induction through stereospecific, reversible binding to a cytosolic receptor protein. When XB cells derived from a mouse teratoma are cul-

tured at high density with lethally irradiated 3T3 cells, the addition of TCDD produces a dose-related keratinization response. This effect occurs in the absence of the feeder cells if the teratoma cells are cultured in 3T3-conditioned media. The keratinization produced by TCDD appears to be similar to that which occurs spontaneously when XB cells are plated at low density. XB cells contain the cytosolic receptor and respond to TCDD with a dose-related induction of aryl hydrocarbon hydroxylase activity. The potencies of halogenated aromatic hydrocarbon congeners [dibenzo-p-dioxins, dibenzofurans, biphenyls and azo(xyl)benzenes] to produce keratinization in XB/3T3 system corresponds to their binding affinities for the cytosolic receptor. Nonhalogenated aromatic hydrocarbons, which are agonists for the receptor, also produce a dose-related keratinization in XB/3T3 cultures, whereas unrelated toxins do not. Our results suggest that the keratinization produced by TCDD and congeners in XB/3T3 cultures is mediated by the cytosolic receptor, and that this system provides an in vitro model for the in vivo toxic effects produced by halogenated aromatic hydrocarbons in the epidermis. (41 Refs)

D.

INDUCTION OF MIXED FUNCTION OXIDASES

175. INDUCING POTENCY OF ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN HUMAN LYMPHOBLASTOID CELLS AND MICE BY POLYCHLORINATED DIBENZOFURAN CONGENERS

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Environ Health Perspect: 59:107-112 1983

Aryl hydrocarbon hydroxylase (AHH)-inducing potency of eight polychlorinated dibenzofuran (PCDF) isomers, 3,4,5,3',4',5'-hexachlorobiphenyl (HCB) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in two inbred mouse strains (AHH responsive and nonresponsive mouse strains) and eight human lymphoblastoid cell lines (four males and four females) was investigated to evaluate their relative toxic potency. In AHH nonresponsive DBA mouse strain, only TCDD induced hepatic AHH activity at a dose of 30 micrograms/kg, while in AHH responsive C57 mouse strain, six PCDF isomers besides TCDD could enhance the enzyme activity significantly. 2,3,7,8-Tetrachlorodibenzofuran (2,3,7,8-TCDF), 1,2,3,7,8-pentachlorodibenzofuran (1,2,3,7,8-PCDF) and 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PCDF) showed the highest AHH inducing activity among the PCDF isomers tested. In contrast with the results obtained from the mouse experiments, in human lymphoblastoid cells, 2,3,4,7,8-PCDF, 1,2,3,4,6,7-hexachlorodibenzofuran (1,2,3,4,6,7-HCDF) and 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HCDF) elicited the highest AHH induction and were as potent AHH inducers as TCDD. These observations suggest that toxicities of 2,3,4,7,8-PCDF, 1,2,3,4,6,7-HCDF and 1,2,3,4,7,8-HCDF in human tissues may be comparable to that of TCDD. It was also observed that in both male and female human cell lines, the degree of AHH inducibilities of these compounds were roughly parallel to that of 3-methylcholanthrene, possibly indicating that genetic susceptibility among human population to the toxic compounds are also present similar to those reported among mouse strains.

176. HUMAN DIOXIN-INDUCIBLE CYTOCHROME P1-450: COMPLEMENTARY DNA AND AMINO ACID SEQUENCE

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Science: 238(4855):30-3 1985

Induction of cytochrome P1-450 has been linked to susceptibility to certain chemically induced cancers in mouse and man. Treatment of the human cell line MCF-7 with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) results in high levels of aryl hydrocarbon (benzo(a)pyrene) hydroxylase (P1-450) activity. This cell line was used to isolate a human P1-450 full-length complementary DNA (cDNA) clone. The cDNA is 2566 nucleotides in length, encodes a polypeptide messenger RNA (2.8 kilobases in length), and has a continuous reading frame producing a protein with 512 residues (molecular weight, 58,151). The human P1-450 cDNA and protein are 63 percent and 80 percent similar to mouse P1-450 cDNA and protein, respectively. Whereas the mouse TCDD-inducible P-450 gene subfamily has two members (P1-450 and P3-450), the human TCDD-inducible gene subfamily appears to have only one gene (P1-450).

177. COMPARISON OF ARYL HYDROCARBON HYDROXYLASE AND ACETANILIDE 4-HYDROXYLASE INDUCTION BY POLYCYCLIC AROMATIC COMPOUNDS IN HUMAN AND MOUSE CELL LINES

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Child Health and Human Development, Bethesda, MD 20205
Biochem Pharmacol: 36(11):2721-31 1983

The human MCF-7 and the mouse Hepa-1 cell culture lines were compared for aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase inducibility by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo(a)anthracene (BA) and TCDD- and BA-specific binding in the cytosol and nucleus. The effective concentration of BA in the growth medium required to induce either enzyme to 50% of its maximally inducible activity (EC50) was the same (5-11 microM) in both MCF-7 and Hepa-1 cells. On the other hand, the EC50 for TCDD in MCF-7 cells (5-25 nM) was more than 40-fold greater than that in Hepa-1 cells (0.4 to 0.6 nM). P1-450- and P3-450-specific mouse cDNA probes were used to quantitate mRNA induction in the Hepa-1 cell line. P1-450 mRNA was induced markedly by TCDD and benzo(a)anthracene, whereas P3-450 mRNA was induced negligibly. A P1-450-specific human cDNA probe was used to quantitate P1-450 mRNA induction in the MCF-7 cell line. Aryl hydrocarbon hydroxylase inducibility by TCDD or BA always paralleled P1-450 mRNA inducibility in either the mouse or human line. Although the cytosolic Ah receptor in Hepa-1 cells was easily detected by sucrose density gradient centrifugation, gel permeation chromatography, and ion-exchange high-performance liquid chromatography, the cytosolic receptor cannot be detected in MCF-7 cells. Following in vivo exposure of cultures to radiolabeled TCDD, the intranuclear concentration of inducer-receptor complex was at least fifty times greater in Hepa-1 than MCF-7 cultures. The complete lack of measurable cytosolic receptor and almost totally absent inducer-receptor complex in the nucleus of MCF-7 cells was, therefore, out of proportion to its capacity for aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase inducibility. This MCF-7 line should provide an interesting model for a better understanding of the mechanisms of drug-metabolizing enzyme induction by polycyclic aromatic compounds, including the Ah receptor-mediated mechanism.

178. METABOLISM OF 2-ACETYLAMINOFLUORENE IN ISOLATED RABBIT PULMONARY CELLS: EVIDENCE FOR THE HETEROGENEOUS DISTRIBUTION OF MONOOXYGENASE ACTIVITY IN LUNG TISSUE

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Drug Metab Dispos: 13(4):406-11 1985

The region-specific hydroxylation of 2-acetylaminofluorene (2-AAF) has been used to monitor monooxygenase activity in isolated rabbit lung cells. Following isolation, the cells were separated into seven different fractions according to size by centrifugal elutriation. Macrophages were recovered from the lungs by lavage and examined in parallel with the parenchymal cell populations. The resulting fractions were assayed for 2-AAF hydroxylase activity and were examined for the presence of endothelial cells (angiotensin-converting enzyme), alveolar type II cells (modified Papanicolaou stain), polymorphonuclear leukocytes (modified Papanicolaou stain), bronchiolar Clara cells (nitro blue tetrazolium stain), and ciliated cells (phase-contrast microscopy). Highest hydroxylase activities were seen in the cell fraction containing the largest percentage of Clara cells. The activity profiles provided evidence for a population of cells not correlating with either alveolar type II cells or Clara cells possessing substantial monooxygenase activity. The alveolar macrophage almost exclusively hydroxylated 2-AAF in the 9-position and the cell fraction containing the higher percentage of endothelial cells metabolized 2-AAF the least. Pretreatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin preferentially induced the 7-hydroxylation of 2-AAF and either did not alter or decreased the rate of formation of the other hydroxy metabolites. An exception was seen in fraction 1 where 2,3,7,8-tetrachlorodibenzo-p-dioxin induced the hydroxylation of 2-AAF to all products except 3-hydroxy-AAF. Evidence is presented for the presence of a cell type(s) not identifiable as either the Clara or alveolar type II cell with considerable monooxygenase activity.

179. CYTOCHROME P-450 MONOOXYGENASE SYSTEM. LOCALIZATION IN SMOOTH MUSCLE OF RABBIT AORTA

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Mol Pharmacol: 28(1):72-9 1985

Cytochrome P-450 monooxygenase isozymes and NADPH-cytochrome P-450 reductase were detected in the microsomal fraction of rabbit aorta by immunoblotting and by enzymatic activity. The monomeric molecular weights of aortic proteins that cross-reacted with antibodies to cytochrome P-450 forms 2 or 6 and reductase were identical to those of the proteins purified from the liver. The induction of form 6 immunoreactive protein and O-deethylation of 7-ethoxycoumarin (a reaction catalyzed by form 6) was observed in aorta following treatment of rabbits with 2,3,7,8-tetrachlorodibenzo-p-dioxin or beta-naphthoflavone. The amount of reductase protein (equivalent to 22.4 +/- 3.2 activity units/mg of protein) correlated with the cytochrome c reductase activity (18.3 +/- 1.8 units/mg) and was the same for both treated and untreated rabbits. Consistent with immunoblot data, the amount of form 2 was insufficient for detection of activity (N-demethylation of benazepamine). Significantly, removal of the endothelium, which was confirmed by light microscopy and by scanning electron microscopy, reduced by only 8 to 32% the specific enzymatic activity or content of immunoreactive proteins; only traces of protein or activity were recovered in the

endothelial fraction. In studies of the vasculature, the potential of this metabolic pathway for the activation or detoxication of mutagens, carcinogens, toxins, or drugs and metabolites of endogenous substrates warrants consideration, especially in regard to the mucosal events reported to be involved in the formation of atherosclerotic plaques.

180. INDUCTION OF P-450 MRNAs AND P-450-MEDIATED ACTIVITIES IN HUMAN BREAST CARCINOMA MCF-7 AND MOUSE HEPATOMA HEPA-1 CELL LINES BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) (MEETING ABSTRACT)

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Fed Proc. 43(4):872 1984

Polycyclic aromatic compounds such as TCDD induce P-450-mediated monooxygenase activities in many mammalian cell culture lines. In order to identify human cell lines suitable for isolation of induced P-450 mRNA, we studied several breast carcinoma cell lines. In MCF-7 cultures, TCDD induces aryl hydrocarbon hydroxylase (AHH) and acetanilide 4-hydroxylase (Ac4H) activities, which are known to be associated in mouse liver with P1-450 and P3-450, respectively. The effective TCDD concentration needed to induce half maximal activity (EC50) in mouse hepatoma Hepa-1 cultures is 0.56 nM for AHH and 0.45 nM for Ac4H; the EC50 for TCDD in MCF-7 cultures is 17 nM for AHH and 5.6 nM for Ac4H. By dot-blot analysis with mouse P1-450 and P3-450 cDNA clones and human P1-450 and P3-450 genomic clones, quantitative mRNA data confirmed the more than 10-fold higher EC50 values for AHH and Ac4H induction by TCDD in the MCF-7 line than those in the Hepa-1 line. Hepa-1 cells contain an Ah receptor with an apparent Kd less than 1.0 nM; when MCF-7 are treated in vitro with 20 nM [³H]TCDD, however, no saturable Ah receptor is detected on sucrose density gradients. Hence, the MCF-7 line provides an interesting model system for studying TCDD action in a cell that lacks a high-affinity Ah receptor.

181. METABOLIC PROCESSING OF 2-ACETYLAMINOFLUORENE BY MICROSOMES AND SIX HIGHLY PURIFIED CYTOCHROME P-450 FORMS FROM RABBIT LIVER

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Carcinogenesis. 5(12):1717-23 1984

Kinetic analysis of oxidative metabolism of 2-acetylaminofluorene (AAF) was studied in control and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced microsomes and with six highly purified cytochrome P-450 isoenzymes from rabbit liver. Kinetic parameters were defined for 7-, 5-, 3-, 1- and N-hydroxylations of AAF. 7-Hydroxylation was best defined by a two enzyme system, displaying a high affinity and relatively low capacity and a low affinity high capacity components in both control and TCDD induced microsomes. All the purified cytochrome P-450 isoenzymes were capable of catalyzing the 7-hydroxylation of AAF and, with the exception of form 4, this was the only oxidation on the AAF molecule catalyzed by these forms. It is probable that forms 1, 4 and 6 accounted for a substantial part (greater than or equal to 25%) of total metabolic capacity corresponding to the high affinity component of 7-hydroxylation, whereas forms 3b and 3c accounted for less than 5% of the metabolic capacity displayed by the low affinity component in control microsomes. However, forms 4 and 6 could account for greater than 90% of the metabolic capacity of the high affinity com-

ponent of 7-hydroxylation in TCDD microsomes, whereas the form(s) responsible for the metabolic capacity of the low affinity component were not identified. Each of the 1-, 3-, 5- and N-hydroxylations were best defined by a single enzyme system in both control and TCDD microsomes (3- and 5-hydroxylations could not be defined in TCDD microsomes). Close agreements were found between the apparent Km for N-hydroxylation in control, TCDD induced microsomes and with form 4. alpha-Naphthoflavone inhibited AAF N-hydroxylation to a similar extent in control and TCDD microsomes and in form 4. These data indicate that a subpopulation of cytochrome P-450 isoenzymes, which includes all the purified P-450 forms tested in the present study, is solely involved in detoxification (i.e., 7-hydroxylation) of AAF, and as such probably behave as a functional unit in vivo; modulation of cytochrome P-450 content by inducers such as TCDD results in emergence of relatively few cytochrome P-450 isoenzymes that can account for most of the oxidative metabolism of AAF; and a single cytochrome P-450 isoenzyme (i.e., form 4) is responsible for catalyzing N-hydroxylation of AAF, the first and the obligatory step in the metabolic activation of this carcinogen.

182. BENZ[a]ANTHRACENE AND 3-METHYLCHOLANTHRENE INDUCTION OF CYTOCHROME P-450 IN C3H/10T1/2 MOUSE FIBROBLASTS. MODULATING ROLE OF CYTOTOXIC 3-METHYLCHOLANTHRENE METABOLITES

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Mol Pharmacol. 23(1):198-205 1983

The relative abilities of benz[a]anthracene, 3-methylcholanthrene (3MC), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to induce aryl hydrocarbon hydroxylase (AHH) in C3H/10T1/2 mouse fibroblasts were studied. Benz[a]anthracene (13 uM, 24-hr exposure) was found to be the most effective inducer of the three compounds; it elicited an 8- to 11-fold increase in AHH activity. No induction was seen after exposure of the cells to 13 uM 3MC, and exposure to 0.1 uM TCDD elicited only a two-fold increase in AHH activity. The rank order of inducible AHH levels in C3H mouse liver microsomes was exactly reversed when these same three compounds were administered to intact C3H mice, the strain from which these C3H/10T1/2 cells were originally derived. In C3H/10T1/2 cell cultures, increasing concentrations of 3MC were able to suppress totally the induction seen with 13 uM benz[a]anthracene alone in a dose-related manner (3MC ID50 = 0.26 uM). A significantly higher concentration of 3MC was required to suppress induction when 26 uM benz[a]anthracene was added to the medium (3MC ID50 = 0.56 uM). The extent to which these ligands compete at the Ah receptor required for AHH induction was examined by sucrose density gradient analysis of a [³H]TCDD receptor in C3H/10T1/2 cell and C3H liver cytosols. Stereospecific, saturable [³H]TCDD receptors were found in both cytosol fractions, and in both cases a 100- to 130-fold excess of benz[a]anthracene, 3MC, or tetrachlorodibenzo-p-dioxin totally abolished [³H]TCDD binding. Cytotoxicity studies revealed more than 90% loss of viability in cells treated with 13 uM 3MC. Partial inhibition by carbon monoxide of the cytochrome P-450 oxidation of 3MC in the cells resulted in a three-fold induction in AHH by 3MC. These data indicate that 3MC binds to the Ah receptor in C3H/10T1/2 cells and can act as an inducer of AHH activity. However, under the normal circumstances in which 3MC metabolism is not suppressed by carbon monoxide, 3MC is converted to a cyto-

toxic derivative(s) which kills the cells and thus prevents a normal induction response. (Author abstract) (38 Refs)

185. MONOOXYGENASE AND EPOXIDE HYDROLASE REGULATION IN PRIMARY FETAL RAT LIVER CELL CULTURE

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Adv Exp Med Biol, 1984;117:97-98 1982

Presence of cytochrome P450 (CP450) was studied in fetal hepatocyte culture and measurements of aryl hydrocarbon hydroxylase (AHH), ethoxycoumarin deethylase, aldrin epoxidase, and epoxide hydrolase (EH) activities were measured. The level of CP450 content in primary fetal liver cell culture was stable and did not significantly differ from that found in fetal liver used for the culture preparation. High concentration of phenobarbital (PB) induced both AHH and EH activities, whereas benzo(a)anthracene acted preferentially, and 2,3,7,8-tetrachlorodibenzo-p-dioxin selectively on AHH activity. AHH and EH activities were modified by addition of dexamethasone. The enzyme activities were first inhibited and then induced when the corticoid concentration was raised from 10⁻¹⁰ to 10⁻⁴ M. The same shift in the AHH inhibition in vitro by alpha-naphthoflavone (ANF) and metyrapone (MTR) was observed, whether the dexamethasone treatment was carried out in the presence or in the absence of serum. The action of other Δ^4 -steroid steroids on both AHH and EH activities was also studied. The same effect was observed with hydrocortisone; a shift in sensitivity of the control and PB-induced AHH activity in vitro from ANF to MTR, and a fall in the control and PB-induced EH activity. Sex hormones, testosterone (TS) and estradiol (ED) slightly induced the AHH activity and decreased the extent of AHH and EH induction by PB. TS and ED did not decrease the EH activity and did not shift in vitro the sensitivity of either the control or the PB-induced AHH to the inhibitors. According to these observations, glucocorticoids are some of the physiological stimuli controlling the expression of drug metabolizing enzyme activities in the perinatal period of life. (21 Refs)

186. DEVELOPMENTAL ONSET OF MIXED-FUNCTION OXIDASE ACTIVITY IN PREIMPLANTATION MOUSE EMBRYOS

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Proc Natl Acad Sci USA, 78(11):6991-6995 1981

Two-cell embryos, obtained from the C57BL/6N and DBA/2N strains, were cultured in media that supported in vitro differentiation and that contained [³H]benzo(a)pyrene. High-pressure liquid chromatography of the activated intermediates formed during in vitro embryonic development indicated that the onset of polynuclear aromatic hydrocarbon activation coincided with blastocyst formation. Comparison of individual oxygenated intermediates metabolically formed from embryos genetically 'responsive' or 'nonresponsive' to aromatic hydrocarbons revealed significant quantitative differences in the production of dihydrodiol, quinone, and phenolic derivatives. In addition to exhibiting basal mixed-function oxidase activity, blastocysts were also responsive to enzymatic induction when exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. The presence of operative metabolite-detoxifying pathways was also assayed. Enzymatic treatment of water-soluble metabolites with beta-glucuronidase or aryl-sulfatase revealed that neither glucuronic acid conjugates nor sulfate ester derivatives were present. These data, therefore,

provide direct evidence that late preimplantation mouse embryos (day 3 1/2 of gestation) are similar to later developmental stages in having the enzymatic capability for xenobiotic activation and enzyme induction but are dissimilar with respect to their detoxification mechanism(s). Moreover, the ability of preimplantation embryos to activate directly polynuclear aromatic hydrocarbon to bioactive intermediates may be of importance in assessing the ontological susceptibility of the developing embryo to carcinogenic or teratogenic chemicals. (Author abstract) (27 Refs)

185. COMPARATIVE INDUCTION OF ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN VITRO BY ANALOGUES OF DIBENZO-P-DIOXIN

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Food Cosmet Toxicol, 18(6):627-633 1980

The abilities of dibenzo-p-dioxin (DD), a predioxin [4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol], and 23 dioxin analogs to induce aryl hydrocarbon hydroxylase (AHH) activity in rat Reuber hepatoma cell cultures were investigated. Hepatoma cells were incubated for 24 hr, treated with various concentrations of a test compound, and incubated for an additional 72 hr; cells were homogenized, and the AHH activity of the homogenate was determined. Various concentrations of test compounds were used in order to determine the 50% effective dose (ED₅₀) for each compound. The mean ED₅₀ for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was 1.24 picomoles/plate; this value was defined as a relative biologic potency (RBP) of 100, and was used for comparison in order to calculate RBPs for the remaining compounds. In comparison with the value for TCDD, eight DD analogs showed a RBP of greater than 0.2%; seven of these nine compounds have halogen substitutions in the four lateral ring positions. Seven additional DD analogs had RBPs of less than 0.2%, and the remaining eight compounds (including DD and the predioxin) were unable to induce AHH. The potential use of AHH-induction in screening for planar polyhalogenated organic contaminants in food and the environment is discussed. (40 Refs)

186. COMPARISON OF ARYL HYDROCARBON HYDROXYLASE AND EPOXIDE HYDRATASE INDUCTION IN PRIMARY FETAL RAT LIVER CELL CULTURE

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Chem Biol Interact, 32(3):361-375 1980

The activity of aryl hydrocarbon hydroxylase (AHH) and/or epoxide hydratase (EH) is induced in primary fetal rat liver cell culture by benzo(a)anthracene (BA), phenobarbital (PB), cigarette smoke condensate (CSC), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and trans-stilbene oxide (TSO). The response of the two enzymes to the different chemicals varies as follows: (a) AHH is induced by lower concentrations of BA, PB and CSC than those required to significantly induce EH; (b) AHH is selectively induced by TCDD and by low BA concentrations; (c) the kinetics of AHH induction by BA, PB and CSC are faster than that of EH; (d) TSO is a selective inducer of EH. As described earlier for AHH, RNA and protein synthesis and the continuous presence of the inducer are required in the early phases of EH induction. Later, when the EH activity has reached a plateau, intact RNA and protein synthesis is not necessary to maintain the enzyme at its optimal value. The removal of the inducer de-

termines a decay of the EH activity, allowing the estimation of a biological $t_{1/2}$ of about 72 hr. TSO prevents the AHH induction by PB, but not that mediated by BA and CSC. Added together with PB, BA, CSC or PB plus BA, TSO induces the EH activity in a more than additive manner. This effect is only seen after 6 days of continuous treatment. These results indicate that in this tissue culture model, the mechanism of AHH and EH induction can clearly be dissociated. (Author abstract) (36 Refs)

187. CYTOCHROME P-450 IN A CULTURED HUMAN LYMPHOCYTE CELL LINE

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Biochem Pharmacol; 27(22):2639-2662 1978

Aryl hydrocarbon hydroxylase (AHH) activity and inducibility were studied in a cultured human lymphocyte cell line, RPMI-1788. The cells were incubated for 48 hr before addition of one of the following AHH inducers: dibenzanthracene (DBA: 0.3 μ M), benzoanthracene (10.0 μ M), 3-methylcholanthrene (MC: 1.0 μ M), or 2,3,7,8-tetrachlorodibenzo-p-dioxin (0.5 nM). AHH activity, measured 24 hr later, had increased 160%-350% over control values. The difference spectra of microsomes isolated from DBA-induced RPMI-1788 cells and from the liver of rats pretreated with phenobarbital (PB) and MC were compared. As in previous reports, difference spectral maxima of liver microsomes from PB- and MC-treated rats were at 450 and 448 nanometers (nm), respectively. The difference spectral max of the RPMI-1788 cell microsomes was at 451 nm. The spectral examination reveals, therefore, that the mixed-function oxygenase-linked cytochrome in RPMI-1788 is distinct from that in rodents. It is suggested that mechanistic, physical, and biochemical information obtained from studies with rodent liver cytochromes P-450 may not be directly applicable to humans. (14 Refs)

E.

OTHER METABOLIC EFFECTS

188. 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) MODULATES EPIDERMAL GROWTH FACTOR (EGF) BINDING TO BASAL CELLS FROM A HUMAN KERATINOCYTE CELL LINE

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Toxicol Appl Pharmacol; 82(3):481-52 1986

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) modulates the proliferation, differentiation, or both, of epidermal keratinocytes in vivo and in culture. The growth of epidermal cells in culture is regulated by several biochemical mediators including epidermal growth factor (EGF). In this report the actions of TCDD on EGF binding in a basal cell population from a human keratinocyte cell line were examined. TCDD decreased the specific binding of ^{125} I-EGF to basal cells by 40% within 96 hr. This reduction in EGF binding could not be attributed to changes in the state of differentiation as assessed by cell size and morphology, and cornified envelope competence, a marker of terminal differentiation. Modulation of EGF binding by TCDD was concentration-dependent ($EC_{50} = 1$ nM) and stereospecific, suggesting involvement of the Ah receptor. Scatchard analysis of EGF binding to the basal cells indicated a single class of high-affinity sites in both control ($K_d = 0.14$ nM) and treated ($K_d = 0.11$ nM) cultures and confirmed a decrease in the number of these sites in response to TCDD. The reduction in EGF binding correlated with a decrease in EGF-stimulated DNA synthesis and cell proliferation. Comparison of differentiation-competent

squamous cell carcinoma (SCC) lines treated with TCDD supported an association between modulation of EGF binding and enhanced differentiation. The data indicate that basal cells are a target for TCDD. We propose that the modulation of EGF binding in basal keratinocytes by TCDD is one of the critical regulatory events resulting in enhanced differentiation.

189. FURTHER CHARACTERIZATION OF TUMOR-PROMOTER-MEDIATED ACTIVATION OF PROTEIN KINASE C

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Biochem Biophys Res Commun; 121(2):448-55 1984

Tumor promoting phorbol esters are able to activate Ca^{2+} -sensitive, phospholipid-dependent protein kinase (protein kinase C) in a reconstituted system. Indol alkaloid telavancin, a tumor promoter, has been found to be as potent as tumor promoters from the series of phorbol esters and mezerein in activating the mouse brain enzyme. Chemically unrelated tumor promoters such as tetrachlorodibenzo-p-dioxin, anthracene and phenobarbital are devoid of effect. Tri- α -glycerol 1,2 dicolein strongly activated the enzyme whereas 1,3 dicolein like 1,2 diisomer were poor activators and 1,5 diisomer was inactive. Although tumor-promoter or diacylglycerol-mediated activation of protein kinase C was observed in the presence of 0.5 mM EGTA, the reaction requires traces of Ca^{2+} . Tumor promoters did not prevent inhibitory action of antiproliferative phenothiazines and local anesthetics but appear to increase IC_{50} of these drugs.

190. INDUCTION OF CYTOSOLIC ALDEHYDE DEHYDROGENASE BY TCDD IN CULTURED HEPATOMA CELLS (MEETING ABSTRACT)

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New Eng. Coll. Osteopathic Med., Bridgford, ME 04605
Fed Proc; 43(7):1956 1984

Exposure of various lines of cultured hepatoma cells to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) elevates cytosolic aldehyde dehydrogenase (ALDH) substantially. Of the cells tested, Rarther H-35 cells demonstrated the greatest overall response, with induced levels more than 100 fold greater than basal levels after 24 days. MH1C1 and HTC cells were also tested and each exhibited substantial induction, although neither line survived longer than 5 days after serum withdrawal. In H-35 cells, ALDH induction required both protein and RNA synthesis. The half-life of ALDH was found to be 16 hr during all phases of induction, hence changes in synthesis appear to be responsible for this effect of TCDD. The time-course was biphasic. Initial equilibrium occurred at 48-72 hr and was followed by resurgent profound elevation through day 26. These results suggest that at least 2 separate biochemical controls are elicited by TCDD exposure. One-day TCDD exposure was sufficient to initiate enzyme induction for the full 24 day time-course. Cell and medium transfer experiments demonstrated that the dioxin was essentially depleted from the medium and sequestered by the cells.

191. CARBARYL BINDS TO PROTEINS OF HUMAN CELLS IN CULTURE BUT CHLORINATED ORGANIC CHEMICALS DO NOT

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Int. Physical and Chemical Res., Wako-shi, Saitama 351, Japan
Bull Environ Contam Toxicol; 28(4):500-505 1982

Radiolabeled carbaryl became highly bound to protein isolated from cultured human embryonic lung diploid cells (HEL 299), whereas benzo(a)pyrene showed less binding. No bind-

ing of the following chlorinated chemicals was detected: 2,4-dichlorophenoxy-3-14C-acetic acid, dichloro-3-U-14C-phenyl-trichloroethane, hexachlorobenzene-14C(U), or polychlorinated biphenyl-14C(U). (10 Refs)

192. INFLUENCE OF INDUCERS OF MONOOXYGENASES ON CYTOTOXIC EFFICIENCY OF ELLIPTICINE ON LEUKEMIA L1210 CELLS

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JNCI: 67(4):871-876 1981

The effects of some inducers of microsomal cytochrome P-450-dependent monooxygenases on the metabolic bioactivation and the cytotoxicity of the antitumoral drug ellipticine (ELPT) were studied. Rate of growth of leukemia L1210 cells was measured *in vitro* in the absence and presence of ELPT or measured when the ELPT was metabolically transformed by noninbred Sprague-Dawley rat liver microsomes. The animals used were either untreated or pretreated by various inducers such as phenobarbital, 3-methylcholanthrene, benzo(a)pyrene, 2,3,7,8-tetrachlorodibenzo-p-dioxin, Aroclor 1254, or ELPT. The transformation of ELPT into its two main metabolites, 9-hydroxyellipticine (9-OHE) and 7-hydroxyellipticine, was studied and measured by high-pressure liquid chromatography in conjunction with the determination of cytotoxic activity. A large variability was observed in the bioactivation and cytotoxic efficiency of ELPT mediated by the different microsomal preparations. The more P-448 and/or P-1450 forms of cytochrome were induced, the more the 9-OHE was produced and the more the cytotoxicity toward L1210 cells was enhanced. These features were compared with those elicited by the activation of cyclophosphamide, which was transformed into cytotoxic metabolites by the cytochrome P-450 form specifically induced by phenobarbital-type inducers. (Author abstract) (27 Refs)

193. PERSISTENT PESTICIDES AND ENVIRONMENTAL CHEMICALS ARE TAKEN UP TO A GREATER EXTENT THAN NON-PERSISTENT COMPOUNDS BY CULTURED HUMAN CELLS

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Zell Environ Contam Toxicol: 19(4):423-437 1978

Data on the cellular uptake of 11 pesticides by cultured human embryonic lung diploid cells are presented. The pesticides dichlorodiphenyltrichloroethane (DDT), dieldrin, aldrin, 2,4,5,2',5'-hexachlorobiphenyl (PCB), hexachlorobenzene (HCB), carbaryl, malathion, parathion, chlordane, 2,4,5-trichlorophenoxy-1-acetic acid (2,4,5-T), and 2,4-dichlorophenoxy-1-acetic acid (2,4-D) showed the following uptake levels (expressed as picomoles/mg cell protein) at 48 hr of incubation: 4,350, 4,380, 3,640, 1,740, 778, 945, 180, 22.6, 32.4, 9.9, and 3.7, respectively. Thus, persistent pesticides such as DDT, dieldrin, aldrin, PCB, and HCB were taken up by cells to a greater extent than nonpersistent pesticides. The considerable amount of carbaryl taken up by the cells suggests that the naphthalene ring in the carbaryl molecule may be involved in the incorporation potential of this compound. The effect of the above pesticides on cell growth was also investigated; most compounds were not toxic at the same concentration (4×10^{-6} M) and over the same experimental period as in the uptake experiments. The herbicides 2,4-D and 2,4,5-T, although less incorporated than the insecticidal and industrial chemicals, appeared to be more toxic. It is evident that differences in uptake are not the cause of the different degrees of cytotoxicity of the chemicals. (13 Refs)

F.

MODULATION OF TOXIC AND METABOLIC EFFECTS

194. PATTERN OF STEROID INTERACTION WITH INDUCERS OF ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN SEVERAL CLONED ESTRADIOL-RESPONSIVE STROMAL CELL STRAINS FROM MOUSE ENDOMETRIUM

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J Cell Physiol: 123(3):395-400 1980

Several steroid responsive stromal cell strains were isolated from normal mouse endometrium. Both estrogen responsive and progesterone responsive strains were obtained. The estrogen responsive cells were also responsive to diethylstilbestrol (DES). The progesterone responsive strains were inhibited by estrone and estradiol. One of the estrogen responsive cell strains, E041, was cloned in the presence and absence of 17-beta-estradiol (E2). Of 14 clones developed in the absence of E2 and screened for responsiveness of growth, six were significantly responsive to E2. Six of 76 clones developed in the presence of E2 were responsive to E2. Microsomes isolated from these clones were shown to have aryl hydrocarbon hydroxylase (AHH) activity which was inducible with either benzo(a)anthracene (BA) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The basal levels of AHH activity were significantly reduced by E2 in E2 responsive clones. The TCDD induced levels of AHH activity in the E2 responsive and E2 non-responsive clones were significantly reduced by E2. The BA induced levels of AHH were significantly increased by E2 in one of the clones. These results show that mouse endometrial stroma consist of a number of populations of cells with different specific proliferative and metabolic responses to steroids.

195. OPPOSING EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND HYDROCORTISONE ON GROWTH AND DIFFERENTIATION OF CULTURED MALIGNANT HUMAN KERATINOCYTES

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Carcinogenesis: 5(1):67-71 1984

The human keratinocyte line SCC-13, derived from a squamous cell carcinoma of epidermis, was examined for effects on growth and differentiation upon treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Inhibition of growth was observable at 0.1 nM and maximal in the concentration range of 1-100 nM TCDD, but was completely antagonized by addition of hydrocortisone to the growth medium. TCDD was found to inhibit several aspects of keratinocyte differentiation that are stimulated by hydrocortisone. In confluent cultures, accumulation of keratin protein and transglutaminase activity were suppressed as well as spontaneous envelope formation and envelope competence. This phenomenon occurred without significant effect of TCDD on depletion of hydrocortisone from the medium. We conclude that the response of SCC-13 cells to TCDD depends upon hormonal conditions in culture and that this agent can interfere with cellular responses to normal physiological conditions, thereby altering the differentiation program ordinarily observed.

196. INDUCTION OF BENZO(A)PYRENE METABOLISM BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES: REGULATION BY VITAMIN A

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Dis Adv Int JBL 43(4):1067-8 1982

To develop a cellular model for studying mechanisms of enzyme induction and the effects of this induction on neoplastic metabolism and cytotoxicity, the induction of benzo(a)pyrene (BaP) metabolism by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was investigated in primary hepatocyte cultures prepared from adult male rats and maintained in chemically-defined medium containing hormones. A derepression of induction was observed during the first 3 days in culture. Mixed function oxidase (MFO) induction by TCDD was inhibited by actinomycin D in parallel with the inhibition of RNA synthesis, suggesting that induction was dependent on RNA synthesis. Addition of 0.8-2.0 mg/ml of retinol acetate (RA) prevented about half of the derepression of induction occurring between 36 and 60 hr in culture. In the absence of serum and RA the rate of protein synthesis increased 1.5 fold between 36 and 60 hr in culture. Addition of either RA or serum prevented this increase in protein synthesis with time in culture. The derepression of MFO induction in primary adult hepatocyte cultures may occur partly as a result of a deficiency of retinol. RA is hypothesized to slow the time course of induction by reducing the rate of protein turnover. RA may also partially block the shift in the dose-response curve for induction by TCDD by maintaining the normal metabolic regulation of the cytosolic receptor for TCDD. Addition of a physiological level of RA to the culture medium may therefore help to maintain the hepatocytes at a level of genetic expression more nearly representative of the intact liver. (no Refs)

197. EXPERIMENTAL BASES FOR APPLICATION OF RETINOIDS IN PROPHYLAXIS AND THERAPY OF BRONCHOGENIC CARCINOMA

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Verh Dtsch Ges Inn Med. 87(1):1080-1085 1981

The prophylactic and therapeutic value of retinoids was studied in vitro by means of the benzo(a)pyrene (BaP) model in human fibroblasts. BaP was metabolized to water-soluble products by an enzyme. This enzyme was maximally induced with 2,3,7,8-tetrachlorodibenzo-p-dioxin. At constant concentrations of the inducer, enzymatic metabolism of BaP decreased with increasing concentrations of vitamin A palmitate or all-trans-retinoic acid (ATRA). Binding of intermediate metabolic products of BaP to DNA decreased in proportion to the level of retinoids. Retinoids evidently reduce carcinogenesis by limiting the activation of carcinogenic compounds such as BaP before cellular transformation occurs. The effect of retinoids on transcribed cells was studied using tissue extracts from adenocarcinoma and squamous-cell carcinoma of the lungs and normal lung tissue. Cells were cultured in the presence of ATRA. In adenocarcinoma extracts ATRA bound to a low-mol wt protein and to albumin. Binding was reversible and specific, suggesting that adenocarcinoma cells have receptors for retinoids. The evidence suggests that retinoids have value in prophylaxis of carcinogenesis, particularly for bronchogenic carcinoma. Such value, however, depends on the development of appropriate analogs without toxic effects. ATRA may be of therapeutic value for tumors that have specific retinoid receptors. (19 Refs)

198. RETINOIDS INHIBIT 2,3,7,8-TETRACHLORODIBENZO-P-DIOXINE-INDUCED ACTIVITY OF BENZO(A)PYRENE METABOLIZING ENZYMES IN HUMAN DIPLOID FIBROBLASTS

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Carcinogenesis, 1(9):733-737 1980

The effect of retinoids on the induction of hydrocarbon metabolizing enzymes by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was studied in human fibroblast cultures. Cells were incubated for 14 hr in the presence of various concentrations of TCDD, TCDD plus vitamin A, or TCDD plus all-trans-retinoic acid (RA); the activity of the hydrocarbon metabolizing enzymes was evaluated by measuring the formation of water-soluble benzo(a)pyrene (BP) metabolites after an additional 12-hr incubation in the presence of 3H-BP. Pretreatment of fibroblasts with TCDD alone produced a dose-dependent increase in the formation of water-soluble BP metabolites; maximal values were obtained at a concentration of 2.5 nM TCDD. When fibroblasts were pretreated with TCDD plus vitamin A or RA, a dose-dependent inhibition of the TCDD-induced increase in BP metabolism was observed. RA was a more potent inhibitor than vitamin A, and the inhibitory effects of the retinoids were completely reversed after they were removed from the cultures. Analysis of results obtained using various concentrations of both TCDD and vitamin A suggested that this retinoid acted through non-competitive inhibition of TCDD. In order to examine the effect of retinoids on the activity of pre-existing BP metabolizing enzymes, fibroblasts were pretreated with TCDD and then treated with 3H-BP, RA, cycloheximide, and actinomycin D. Under these conditions, RA reduced the production of water-soluble BP metabolites to 2/3 of the extent obtained by TCDD alone; when fibroblasts were treated concomitantly with RA and TCDD, RA reduced the production of water-soluble BP metabolites to 1/10 of the extent obtained by TCDD alone. (22 Refs)

V.

INTERACTION OF DIOXINS AND DIBENZOFURANS WITH RECEPTORS

A.

GENERAL

199. CARCINOGEN-BINDING PROTEINS IN THE RAT VENTRAL PROSTATE: SPECIFIC AND NONSPECIFIC HIGH-AFFINITY BINDING SITES FOR BENZO(A)PYRENE, 3-METHYLCHOLANTHRENE, AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Cancer Res. 46(2):651-7 1986

The polychlorinated dibenzodioxin [3H]-2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and the carcinogens [3H]benzo(a)pyrene and [3H]-3-methylcholanthrene bound to saturable binding sites in cytosol from the rat ventral prostate. Analysis of equilibrium binding parameters in diluted cytosol preparations indicated an apparent Kd of approximately 2 nM and a binding capacity of approximately 1 nmol/mg cytosolic protein, corresponding to approximately 5% of the total protein content. However, gel permeation chromatography analysis as well as velocity sedimentation

analysis on sucrose gradients of [^3H]TCDD-labeled rat prostatic cytosol indicated binding of [^3H]TCDD to two discrete species. These analyses indicated a sedimentation coefficient of 3.6-3.8S, a Stokes radius of 25-28 Å, and a calculated relative molecular weight of 42,000-45,000 for the most abundant binding species. The other binding species sedimented at 4-5S under high ionic strength conditions and at 8-10S under low ionic strength conditions and had a Stokes radius of approximately 60 Å, a relative molecular weight of approximately 100,000 and an estimated concentration of 5-20 fmol/mg cytosolic protein. Binding of [^3H]TCDD to this species was displaceable by a 200-fold M excess of 2,3,7,8-tetrachlorodibenzofuran. Therefore, this species was tentatively identified as the TCDD receptor. The properties of the high-capacity binder of [^3H]TCDD were found to be similar to the characteristics of a protein previously purified from the rat ventral prostate, prostatic secretory protein, which binds androgens as well as estramustine, a nitrogen mustard derivative of estradiol. The binding of estramustine to diluted prostatic cytosol was shown to be competitively inhibited by 2,3,7,8-tetrachlorodibenzofuran. Moreover, purified prostatic secretory protein bound [^3H]TCDD, [^3H]benzo(a)pyrene, as well as [^3H]-3-methylcholanthrene. It is suggested that binding to this protein is responsible for the high-binding capacity of carcinogens in cytosol from the rat ventral prostate.

200. MECHANISMS OF THE BIOLOGICAL EFFECTS OF PCBs, POLYCHLORINATED DIBENZO-P-DIOXINS AND POLYCHLORINATED DIBENZOFURANS IN EXPERIMENTAL ANIMALS

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Environ Health Perspect. 60:41-46, 1983

Polychlorinated biphenyls, certain polychlorinated dibenzo-p-dioxins and certain polychlorinated dibenzofurans cause a variety of biological effects in experimental animals. The mechanism of the induction of certain enzymes is perhaps best understood. That is, there is binding of certain chlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans to a receptor, translocation of the compound-receptor complex into the nucleus followed by an increased activity of a number of enzymes in the cell. Although the concentration of this receptor in various tissues of some mouse strains correlates well with the intensity of some of the biological effects observed in the mouse strains exposed to these compounds, this correlation apparently does not extend across various species. The current evidence suggests that the acute toxic effects of TCDD in various species is in some way associated with binding of TCDD to the receptor. However, biological effects of TCDD in addition to those resulting from binding to the receptor may be required to produce acute toxicity and, perhaps, other effects. The acute toxic effects of TCDD are probably caused by the parent compound rather than metabolites; however, this conclusion must be viewed as tentative. Also, it cannot be excluded at this time that biological effects other than acute toxicity may be caused by metabolites of TCDD. Finally, the acute toxic effects of TCDD appear not to be related, at least not directly, to the rate of metabolism of TCDD in experimental animals nor to the half-life of excretion.

201. MECHANISM OF ACTION OF TOXIC HALOGENATED AROMATICS

Vickery AE, Sleep TC, Luster GW

Environ Health Perspect. 59:121-129, 1985

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related halogenated aromatic hydrocarbons are a highly toxic class of environmental contaminants, as evidenced by numerous cases of accidental poisonings of human and animal populations

and their extreme toxic potency in laboratory animals. The proposed model for the mechanism of action of TCDD and related compounds is analogous to that of the steroid hormones, which modulate gene expression through a receptor mechanism. In the steroid receptor model, the compound enters the cell cytoplasm where it acts as a specific ligand, binding selectively to a high affinity receptor protein. Bound to the appropriate ligand, the receptor concentrates in the nucleus where its increased association with chromatin leads to altered gene expression. This model has been useful in characterizing the Ah receptor; however, it does not provide a unifying hypothesis for all biochemical and toxic effects associated with exposure to halogenated aromatic hydrocarbons. Several findings suggest that a primary factor in determining TCDD toxicity might be tissue and species specific factors that control the actions of Ah receptor(s) in target tissues. Furthermore, numerous mechanisms might be involved. Clarifying the mechanism(s) for TCDD toxicity would enhance our ability to predict human health consequences to toxic halogenated aromatic hydrocarbons and would provide a more rational basis for risk analysis.

202. A HYDROXYLAPATITE MICROASSAY FOR RECEPTOR BINDING OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND 3-METHYLCHOLANTHRENE IN VARIOUS TARGET TISSUES

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Anal Biochem. 144:213-214, 1985

A 'batch' hydroxylapatite assay for the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) receptor that does not require detergents is described. The receptor could be assayed in rat target tissues using either of the cytochrome P1-450 inducers [^3H]TCDD or [^3H]-3-methylcholanthrene as radioligands. A phosphate buffer washing procedure was developed on the basis of chromatographic data and optimized to separate non-specifically and specifically bound ligand. The assay was characterized with respect to washing efficiency, binding specificity, competition, adsorption time, amount of hydroxylapatite required to bind receptor complexes, sensitivity, and effects of detergents. Equilibrium binding parameters were determined. Receptor extracted with phosphate from hydroxylapatite was analyzed on sucrose gradients and was found to exhibit the same sedimentation properties as the receptor in crude cytosol. Furthermore, the applicability of the assay has been demonstrated in cytosolic preparations from three different target tissues: liver, lung, and thymus.

203. CONTROL OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN BINDING PROTEIN(S) IN THE HAMSTER KIDNEY

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Pharmacology. 30:31-245-54, 1985

Hamster renal cytosol binds [^3H]-2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) with high specificity. Sucrose density gradient centrifugation revealed two binding entities: one with a low sedimentation coefficient of 4-5 S which was displaced by neither TCDD nor other polycyclic aromatic hydrocarbons (PAHs) and another with a high sedimentation coefficient of 7-8 S which was displaced by TCDD, benzo(a)pyrene (BP), 2-methylcholanthrene (MC), and 7,12-dimethylbenzo(a)anthracene (DMBA) but not by estradiol (E), progesterone (P), cortisol (F), testosterone (T), 5 alpha-dihydrotestosterone (DHT), or 17 ethyltestosterone (R-1881).

a synthetic androgen. Cytosol from intact male hamsters showed maximum binding of labelled TCDD to the 7-8 S binding site. Castration or hypophysectomy reduced this binding. Pretreatment with DMBA increased binding, whereas diethylstilbestrol (DES) decreased binding. Sex difference was observed in the binding capacity of renal cytosol. This is the first report of endocrine control over TCDD binding and its modulation by other PAHs and steroids.

204. IDENTIFICATION OF THE AH RECEPTOR IN SELECTED MAMMALIAN SPECIES AND INDUCTION OF ARL HYDROCARBON HYDROXYLASE

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Eur J Biochem. 167(2):629-35 1985

The Ah receptor protein, important in the mechanism of induction of aryl hydrocarbon hydroxylase activity, has been identified and partially characterized in hepatic cytosolic preparations from rat, BALB/c mouse, gerbil, hamster, rabbit, ferret and guinea-pig by means of sucrose density centrifugation analysis and hydroxyapatite binding assays. Using 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as the ligand, total specific binding capacities ranged over 74-691 fmol [3H]TCDD/mg cytosolic protein and apparent dissociation constants ranged over 0.30-7.8 nM. There was no quantitative correlation between the concentration of cytosolic Ah receptors and the 3-methylcholanthrene-mediated induction of aryl hydrocarbon hydroxylase activity in the species studied. Competitive binding studies with a series of monohydroxylated benzo(a)pyrene derivatives suggested the importance of electronic character in their ability to bind to the Ah receptor and to compete with TCDD for specific binding sites on the receptor.

205. EFFECTS OF SUBSTITUENT STRUCTURE ON BINDING TO THE TCDD RECEPTOR: A QSAR ANALYSIS (MEETING ABSTRACT)

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Proc Annu Meet Am Assoc Cancer Res. 26:7 1985

The cytosolic Ah receptor protein plays a role in modulating the biologic, toxic and carcinogenic effects of polynuclear aromatic hydrocarbons and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We have probed the nature of ligand-receptor protein interactions by measuring the binding affinities (EC50) of sixteen 7-X-2,3-dichlorodibenzo-p-dioxins for the rat cytosolic receptor using the competitive displacement assay and [3H]-TCDD as the radioligand. The EC50 value for CF3 analog was 1.95×10^{-8} and was greater than 1000-fold more active than the 7-amino substituted dioxin ($EC_{50} = 2.88 \times 10^{-5}$). Multiple parameter linear regression analysis of the data for fourteen different compounds gave the following equation which showed that the binding affinity was linearly dependent on the hydrophobicity (π) of the 7-X group: $\log(1/EC_{50}) = 1.24 \pi + 3.40$. The effects of substituents (X) on AHH induction in rat hepatoma cells (Eq. 2: $\log(AHH) = 1.29 \log(\text{binding}) + 2.19 \Delta\text{B5} - 1.31 (\Delta\text{B5})^2 - 1.4$) demonstrated the importance of a steric factor (ΔB5) which is consistent with a structure-dependent conformational change(s) in the receptor protein-ligand complex after the initial binding event.

206. LOSS OF THE AH RECEPTOR BINDING PROTEIN IN CYTOCHALASIN B CONTAINING AND GLUCOSE FREE MEDIA (MEETING ABSTRACT)

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Fed Proc. 44(5):1626 1985

Induction of the microsomal cytochrome P1-450 enzyme aryl hydrocarbon is dependent upon binding of polycyclic aromatic hydrocarbons to a specific cytosolic protein termed the Ah receptor. Following binding of xenobiotic compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the cytosolic receptor, the complex then undergoes a temperature dependent process of nuclear translocation resulting in the de novo synthesis of several drug metabolizing enzymes. While performing enucleation experiments with the mouse hepatoma line, Hepa-1, to determine the intracellular localization of this receptor, we observed that the levels of receptor recovered were significantly decreased from expected values. Control experiments to assess the fate of the receptor in the presence of either Ficoll or Cytochalasin B indicated that these two compounds each resulted in an immediate time dependent loss of nuclear and cytosolic receptors from cells. Cytochalasin B is a known inhibitor of glucose transport and its effect on decreasing the intracellular receptor concentration was shown to be mimicked by removing all glucose from the incubation medium. Loss of the TCDD binding protein thus appears to be a specific response to the inhibition of glucose uptake and studies are currently underway to determine if it is due to a reversible inactivation or a selective degradation of receptor.

207. MEASUREMENTS OF THE CYTOSOLIC AH RECEPTOR AMONG FOUR STRAINS OF DROSOPHILA MELANOGASTER

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Arch Toxicol. 58(4):219-25 1985

Four strains of *Drosophila melanogaster* exhibit differences in aryl hydrocarbon hydroxylase (AHH) inducibility by phenobarbital or Aroclor 1254, yet do not show the typical AHH induction response when exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or benzo(a)anthracene. Adult flies were nevertheless examined for the presence of cytosolic TCDD-specific binding (Ah receptor). Berlin-K and Haag 79 exhibit AHH induction by Aroclor 1254 and possess detectable amounts of Ah receptor. Hikone-R has negligible AHH inducibility by Aroclor 1254, yet possesses measurable amounts of the receptor. Oregon-K displays AHH induction by Aroclor 1254 but has no detectable levels of the cytosolic receptor. Specific (high-affinity, low-capacity and saturable) binding of [3H]-1,6-TCDD to the Ah receptor in *D. melanogaster* was shown to be similar to that observed in C57BL/6 mouse liver. Similar specific binding of generally labeled [3H]benzo(a)anthracene in *D. melanogaster* cytosol was not found. These data suggest that the presence of the Ah receptor per se, or quantity of receptor, does not guarantee AHH inducibility by TCDD or benzo(a)anthracene in adults of these four fruit fly strains.

208. COMPARATIVE STUDIES OF ARYL HYDROCARBON HYDROXYLASE AND THE AH RECEPTOR IN NONMAMMALIAN SPECIES

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Comp Biochem Physiol [C]. 80(2):319-24 1985

In vivo treatment of chicks, quail and rats with 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) or 3-methylcholanthrene (MC) caused a dose-dependent increase in hepatic microsomal aryl hydrocarbon hydroxylase activity. A much lower level of AHH induction was observed following similar treatment of trout with high concentrations of TCDD or MC. No induction was apparent in midgut tissues from southern armyworm larvae exposed to the same inducers. A low level of receptor exhibiting specific binding of [^{3} H]TCDD was demonstrated in chick hepatic cytosol, but no evidence of receptor was obtained with the other species. Although the specific binding of the receptor in chick cytosol was only 6-8 fmoles TCDD bound/mg protein compared to 135 fmoles/mg in rat hepatic cytosol, the chick receptor exhibited properties similar to those of Ah receptors in mammals.

209. POLYCHLORINATED DIBENZOFURANS (PCDFs): ENVIRONMENTAL OCCURRENCE, PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES

Van Den Berg M, Olie K, Hutzinger O
Toxicol Environ Chem. 9:171-217 1985

210. CYTOSOLIC RECEPTOR FOR 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN. EVIDENCE FOR A HOMOLOGOUS NATURE AMONG VARIOUS MAMMALIAN SPECIES

Gastmiz T.J., Rust G
Mol Pharmacol 26(1):90-98 1984

The presence and properties of the Ah receptor were examined in the guinea pig, rat, hamster, monkey, and three different strains of mice. These species and strains have demonstrated differences in sensitivity and variability of response to 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds. All species examined, with the exception of DBA/2J mice, possess similar amounts of binding protein with high affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin in hepatic tissue. Numerous dibenzo-p-dioxin congeners and polycyclic aromatic hydrocarbons demonstrated a similar rank order ability to bind to receptor molecules from these species. When analyzed by gel-exclusion high-performance liquid chromatography, hepatic cytosolic receptors from all species eluted at volumes corresponding to a similar molecular weight range. Association of the hepatic Ah receptor with the nuclear fraction was observed in all cases following the i.p. treatment of guinea pig, rat, C57BL/6J mouse, or hamster with [^{3} H]2,3,7,8-tetrachlorodibenzo-p-dioxin. In all species and tissues examined, with the exception of hamster duodenum and thymus, the highest concentrations of receptor were localized in the liver, lung, thymus, intestine, and kidney. Exceptionally high concentrations of receptors were also observed in guinea pig testes. These findings indicate that, despite species and tissue specific differences in the biochemical and toxicological responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds, a number of different mammalian species possess Ah receptors with similar properties. Thus, the correlative differences between certain strains of mice in terms of altered specific binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin and sensitivity to this compound may be unique and not necessarily applicable to other species. Although all data indicate that the receptor mediates these responses, it appears that species- and tissue-specific differences

may be determined by a number of additional factors. These results also suggest the conservation of some, as yet unknown, functional role of the receptor molecule.

211. AH RECEPTOR IN PRIMATE LIVER: BINDING OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND CARCINOGENIC AROMATIC HYDROCARBONS

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Can J Physiol Pharmacol. 62(10):1292-3 1984

Ah receptor in hepatic cytosols from adult cynomolgus monkeys (*Macaca fascicularis*) was identified and quantitated by its binding of the highly toxic chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and the carcinogens 3-methylcholanthrene, benzo(a)pyrene, and dibenz(a,h)anthracene. The concentration of Ah receptor in cynomolgus hepatic cytosols (approximately 10 fmol/mg cytosol protein) was about one-quarter of that typically detected in rodent hepatic cytosols. Receptor concentrations were equal in male and female cynomolgus. [^{3} H]TCDD bound to cytosolic receptor with high affinity (K_d approximately 3 nM). In rodents, Ah receptor is known to play a central role in toxicity caused by halogenated aromatic compounds and in carcinogenesis caused by polycyclic aromatic hydrocarbons. Existence of Ah receptor in monkeys indicates that the receptor also may mediate such responses in primates.

212. BINDING OF BENZO(A)PYRENE AND DIBENZ(A,H)ANTHRACENE TO THE AH RECEPTOR IN MOUSE AND RAT HEPATIC CYTOSOLS

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Cancer Res. 44(4):1426-32 1984

Binding of the polycyclic aromatic hydrocarbon (PAH) carcinogens [^{3} H]benzo(a)pyrene (BP) and [^{3} H]dibenz(a,h)anthracene [DB(a,h)A] to components in rodent hepatic cytosols was characterized by sucrose density gradient centrifugation and by gel permeation chromatography on Sephacryl S-300. In hepatic cytosols from Sprague-Dawley rats, [^{3} H]BP and [^{3} H]DB(a,h)A bound to a component which sedimented at 8 to 9S under low-ionic-strength conditions, and had a Stokes' radius of 4.7 nm. The 8 to 9S component also bound [^{3} H]2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and [^{3} H]3-methylcholanthrene (MC), two compounds previously established as Ah receptor ligands. In hepatic cytosols from C57BL/6J mice [a strain genetically 'responsive' for induction of aryl hydrocarbon hydroxylase (AHH, cytochrome P1-450) by PAHs], [^{3} H]DB(a,h)A bound to a component sedimenting at 8 to 9S which had a Stokes' radius of approximately 6 nm. The 8 to 9S component also bound [^{3} H]TCDD and [^{3} H]MC, but no direct binding of [^{3} H]BP could be detected at 8 to 9S in hepatic cytosol from C57BL/6J mice, nor could specific [^{3} H]BP binding to the 6 nm component be detected in C57BL/6J cytosol by Sephacryl S-300 chromatography. The 8 to 9S component was not detectable in hepatic cytosol from DBA/2J mice (genetically 'nonresponsive' to AHH induction by PAHs) with [^{3} H]BP or [^{3} H]DB(a,h)A, nor with the previously established Ah receptor ligands [^{3} H]TCDD or [^{3} H]MC. In addition to binding to the 8 to 9S component in C57BL/6J cytosols, [^{3} H]BP, [^{3} H]DB(a,h)A, and [^{3} H]MC also bound extensively to a cytosolic component sedimenting at 4 to 5S with a Stokes' radius of approximately 3 nm. The 4 to 5S component was present in hepatic cytosols from genetically nonresponsive DBA/2J mice, as well as in genetically responsive C57BL/6J mice. Binding of

[3H]BP, [3H]DB(a,h)A, and [3H]MC to the 8 to 95 component in rodent hepatic cytosols was eliminated by incubation in the presence of a 100-fold molar excess of nonradioactive TCDD, whereas TCDD had no effect on binding of [3H]BP, [3H]DB(a,h)A, or [3H]MC to the 4 to 55-3 nm component. The 8 to 95 component segregates with the Ahb allele in genetic crosses with mice, but the 4 to 55 component is not associated with the Ahb allele which governs AHH responsiveness. Specificity of chemicals that bind to the 8 to 95 component and segregation of the 8 to 95 component with the Ahb allele identify it as the Ah receptor which functions to regulate AHH induction in animals treated with PAHs. (ABSTRACT TRUNCATED AT 400 WORDS)

213. ELEVATED BINDING OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND 3-METHYLCHOLANTHRENE TO THE AH RECEPTOR IN HEPATIC CYTOSOLS FROM PHENOBARBITAL-TREATED RATS AND MICE

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Biochem Pharmacol. 33(4):531-8 1984

Binding of 3-methylcholanthrene (MC), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and other 'MC-type' inducers to cytosolic Ah receptor sites is the first specific step in induction of aryl hydrocarbon hydroxylase (AHH; cytochrome P1-450) by these compounds. [3H]TCDD and [3H]MC were used as radioligands to quantitate and characterize Ah receptor in hepatic cytosols from genetically 'responsive' C57BL/6J mice, genetically 'nonresponsive' DBA/2J mice, and AHH-inducible Sprague-Dawley rats. Injection of 50-100 mg/kg of phenobarbital (PB) for 3 days more than doubled the concentration of Ah receptor in hepatic cytosol from Sprague-Dawley rats. In C57BL/6J mice, PB injection at 25 mg/kg X 3 days significantly increased (P less than 0.01) the Ah receptor concentration in hepatic cytosol. No cytosolic Ah receptor was detectable in hepatic cytosol from untreated DBA/2J mice, nor did any Ah receptor appear after PB treatment in this nonresponsive strain. Although PB significantly elevated Ah receptor in hepatic cytosols of responsive rodents, many previous studies have shown that the maximal level of AHH activity in animals given PB and an 'MC-type' inducer simultaneously is additive rather than synergistic. Ah receptor concentrations can be doubled by PB treatment without doubling the subsequent AHH-induction response to 'MC-type' compounds. Thus, the cytosolic Ah receptor concentration per se may not be the primary determinant of a given tissue's maximal capacity for AHH induction by 'MC-type' compounds.

214. POLYCHLORINATED DIBENZOFURANS (PCDFs): EFFECTS OF STRUCTURE ON BINDING TO THE 2,3,7,8-TCDD CYTOSOLIC RECEPTOR PROTEIN, AHH INDUCTION AND TOXICITY

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Toxicology. 32(2):151-44 1984

The effects of structure on the activity of 26 polychlorinated dibenzofurans (PCDFs) as competitive ligands for the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) rat hepatic cytosolic receptor protein were determined in a dose-response fashion. The ED50 values for these compounds varied 100,000-fold and the most active PCDFs were substituted in the 2,3,7 and 8 lateral positions; the ED50 for the most active PCDF, 2,3,4,7,8-pentachlorodibenzofuran was 1.5×10^{-8} M which was only slightly less active than 2,3,7,8-TCDD (1.0×10^{-8} M).

A comparison of the binding affinities of several isomer pairs also indicated the relative importance of chlorine substitution at C-4 (or C-6) compared to C-1 (or C-9). Moreover, for some isomers it is apparent that C-4 (or C-6) substituents are more active than lateral substituents for facilitating ligand binding to the receptor protein. This is illustrated by the relative binding potencies of the following isomer pairs: 1,2,4,6,7-/1,2,4,7,8 = 19.2; 2,6,7-/2,3,8 = 2.2; 1,3,6-/1,3,8 = 19. Most of the PCDF structure-activity effects noted above were also observed for the induction of aryl hydrocarbon hydroxylase (AHH) and ethoxycresorufin O-deethylase (EROD) in rat hepatoma H-4-II-E cells in culture. The most active compounds were also substituted in the lateral 2,3,7 and 8 positions and a comparison of C-4 (or C-6) vs. C-1 (or C-9) substituted PCDFs confirmed the higher induction potencies for most of the former group of compounds. The in vitro quantitative structure-activity data were complemented by in vivo studies which determined the relative activities of selected PCDFs as inducers of hepatic microsomal cytochrome P-448 dependent monooxygenases and their effects on body weight gain and thymus weights in immature male Wistar rats. The results indicated that for 2 series of isomers, namely the 2,3,4,7,8- and 1,2,4,7,8- and 1,2,4,7,9-pentachlorodibenzofurans and the 2,3,7,8-, 2,3,4,8- and 1,2,4,8-tetrachlorodibenzofurans, their biologic and toxic potencies were dependent on one major structural factor, the number of lateral chloro substituents. These results support the proposed role of the cytosolic receptor protein in mediating the biologic and toxic effects of the PCDFs.

215. 2,3-DICHLORO-7-AMINODIBENZO-P-DIOXIN (DCADD): A PARTIAL AGONIST FOR THE 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) RECEPTOR IN THE RAT HEPATOMA CELL LINE, H4-II-E (MEETING ABSTRACT)

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The biological responses produced by TCDD, a potent toxin, teratogen and tumor promoter, appear to be mediated by its stereospecific, reversible binding to a cytosolic protein, the TCDD receptor (Poland and Knutson, Ann. Rev. Pharmacol. Toxicol. 22:517, 1982). All congeners studied to date that bind to the receptor have biological efficacies equivalent to that of TCDD (full agonists). The results of experiments on a new congener, DCADD, having receptor affinity but, diminished biological activity are now presented. The binding affinities for TCDD and DCADD, determined in liver cytosol from C57BL/6 mice, were 0.2 nM and 6 uM, respectively. In H4-II-E cells, aryl hydrocarbon hydroxylase (AHH) activity was induced by TCDD and DCADD with EC50's of 0.02 nM and 6 nM, respectively. However, at maximally effective concentrations of DCADD, the fractional response (f) was 0.2 (f = 1.0 for maximally effective concentrations of TCDD). The induction of AHH activity by TCDD was inhibited competitively by DCADD (2 uM). In addition, DCADD (2 uM) produced an equivalent competitive inhibition of AHH induction by 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin, a full agonist 30 times less potent (EC50 0.6 nM) than TCDD. In summary, the results obtained in the present study indicate that DCADD is a TCDD receptor agonist with a reduced biological efficacy (partial agonist) in H4-II-E cells. (1 Ref)

216. INDUCTION OF 7-ETHOXYCOUMARIN O-DEETHYLASE ACTIVITY IN CULTURED HUMAN EPITHELIAL CELLS BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD): EVIDENCE FOR TCDD RECEPTOR

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Biochem Biophys Res Commun, 115(2):611-7 1983

The responsiveness of 3 human squamous cell carcinoma (SCC) lines derived from tumors of the epidermis and tongue to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was assessed by measuring the induction of the cytochrome P1-450-mediated monooxygenase activity, 7-ethoxycoumarin O-deethylase (ECOD). In 4 of the SCC lines the EC50 for this response was approximately 10(-9)M, whereas in one line the EC50 was 10(-10)M. In each of the less sensitive lines a concentration of 10(-10)M TCDD elicited less than 5% of the maximal enzyme activity. Specific binding of radiolabeled TCDD was detected in the cytosol fraction from all the SCC lines. The relative amount of receptor measured in each line correlated with maximally-induced ECOD activity. The data indicate that human cell lines derived from a target tissue for TCDD toxicity contain the TCDD receptor and show differential sensitivity to TCDD analogous to the murine strain differences in sensitivity regulated by the Ah locus.

217. DEFECTIVE BINDING OF 3-METHYLCHOLANTHRENE TO THE AH RECEPTOR WITHIN C3H/10T1/2 CLONE 8 MOUSE FIBROBLASTS IN CULTURE

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Eur J Biochem, 132(2):219-27 1983

C3H/10T1/2 clone 8 mouse fibroblasts (C3H/10T1/2 cells) exhibit induction of aryl hydrocarbon hydroxylase (cytochrome P1-450) when exposed in culture to benzo(a)pyrene, benzo(a)anthracene or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), but do not display the induction response when treated with 3-methylcholanthrene (MCA), the classical inducer of cytochrome P1-450. Induction of cytochrome P1-450 is regulated by the Ah receptor which initially binds inducing chemicals in the cytoplasm, after which the inducer x receptor complex translocates into the nucleus. Cytosolic and nuclear forms of the Ah receptor can be detected in C3H/10T1/2 cells using [3H]TCDD as the radioligand in culture, but specific Ah receptor binding is not detectable within C3H/10T1/2 cells incubated with [3H]MCA. In contrast, in Hepa-1c1 cells, which exhibit cytochrome P1-450 induction when treated with MCA, cytosolic and nuclear Ah receptor can be detected by incubation of the cells either with [3H]MCA or with [3H]TCDD. Nonradioactive MCA is able to compete with [3H]TCDD for Ah receptor sites in C3H/10T1/2 cells, but the relative potency of MCA as a competitor is lower within C3H/10T1/2 cells than in C3H/10T1/2 cells during extracellular incubation. Specific binding of [3H]MCA to Ah receptor can be detected by incubation of [3H]MCA with C3H/10T1/2 cytosol outside the cell. The selective loss of response to MCA as a cytochrome P1-450 inducer (while retaining response to other inducers) appears to be due to defective interaction of MCA with the Ah receptor within the intracellular environment. The specific molecular alteration which makes the MCA x receptor complex ineffective within C3H/10T1/2 cells is unknown. Some fibroblast lines other than C3H/10T1/2 also selectively fail to respond to MCA; thus, this variation in Ah receptor function may not

be due to a mutational change in the Ah regulatory gene which codes for the Ah receptor.

218. COMPOUNDS IN URBAN AIR COMPETE WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN FOR BINDING TO THE RECEPTOR PROTEIN

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Clin Biochem, 66(3):355-66 1983

Acetone extracts of filter-collected urban airborne particulate matter contain compounds which can competitively inhibit 2,3,7,8-[1,6-3H]tetrachlorodibenzo-p-dioxin (TCDD) binding to the rat liver TCDD-receptor protein. The concentration of conventional polycyclic aromatic hydrocarbons (PAHs) or chlorinated dioxins and dibenzofurans cannot account for more than 1-30% of the observed competition for [3H]TCDD binding to the receptor protein. The difference in potency between samples collected in urban areas during different periods of the year and a background sample is 25-400-fold. Collecting samples in the presence of increased concentrations of nitrogen dioxide, nitrous acid, nitric acid or ozone did not increase the amount of compounds with receptor affinity. However, with nitrogen dioxide and especially with nitric acid, a substantial increase of the mutagenic effects in the Ames Salmonella assay in the absence of mammalian activation as well as a degradation of several PAHs were noted. Affinity for the TCDD-receptor protein, mutagenicity in the absence of mammalian metabolic activation in the Ames Salmonella assay and PAH-content are characteristics of urban particulate matter showing the presence of compounds, that represent potential health risks. The compounds with affinity for the receptor may constitute a group of substances different from both conventional PAHs and direct-acting mutagens.

219. COMPETITIVE BINDING TO THE CYTOSOLIC 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN RECEPTOR. EFFECTS OF STRUCTURE ON THE AFFINITIES OF SUBSTITUTED HALOGENATED BIPHENYLS. A QSAR ANALYSIS

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Biochem Pharmacol, 32(24):3803-13 1983

The proposed mechanism of action of the toxic halogenated aromatics, typified by 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD), involves the initial binding to a high-affinity, low-capacity, cytosolic receptor protein. Previous studies have shown that several 4'-halo-2,3,4,5-tetrachlorobiphenyls bind to the TCDD receptor and that a lateral substituent on both phenyl rings is required for activity. Using an extended series of eighteen 4'-substituted-2,3,4,5-tetrachlorobiphenyls as probes, the effects of a variable lateral substituent on receptor binding affinity and the induction of aryl hydrocarbon hydroxylase (AHH) in vivo and in rat hepatoma H-4-II E cells have been determined. For most substituents, there was an excellent correlation between the rank-order potency for receptor binding and the rank-order potency for AHH induction. Based on in vitro binding affinities (EC50 values) of the 4'-substituted tetrachlorobiphenyls, a multiparameter regression equation was formulated correlating the binding constants to physicochemical substituent parameters. For thirteen compounds out of the present series, multiple regression analysis of the binding data led to the following equation: $\log(1/EC50) = 1.53 \text{ sigma} + 1.47 \text{ pi} + 1.09 \text{ HB} + 4.08$, $r = 0.978$. The results suggest that halogen substitution on both phenyl rings is not a requirement for binding and

that hydrophobic (π) and electronic (σ) substituent constants and a variable for hydrogen bond (HB) formation are significant parameters describing relative binding avidities of this series of substituted biphenyls for the TCDD receptor.

220. PHYSICOCHEMICAL CHARACTERIZATION OF SPECIFIC AND NONSPECIFIC POLYAROMATIC HYDROCARBON BINDERS IN RAT AND MOUSE LIVER CYTOSOL

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J Biol Chem, 258(22):13335-42 1983

The aryl hydrocarbon hydroxylase inducers 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene, and benzo(a)pyrene were all shown to bind in a saturable manner to a distinct component in cytosol from both rat and C57BL/6J mouse liver. This component was analyzed by gel permeation chromatography on Sephacryl S-300 and by sucrose density gradient centrifugation and was found to have a Stokes radius of 61 ± 1.5 Å and a sedimentation coefficient of 4.4 S under high salt conditions. Based on these parameters, which were identical for the rat and mouse receptor, a molecular weight of 111,000 was calculated. The same Stokes radius and sedimentation coefficient were observed regardless of the ligand used for labeling of the receptor protein [(3H)TCDD, 3-(3H)methylcholanthrene or 3-(3H)benzo(a)pyrene]. On the other hand, 3-(3H)methylcholanthrene and 3-(3H)benzo(a)pyrene exhibited much more nonspecific binding than 3-(3H)TCDD, at least partially due to contaminating serum components, and it cannot be excluded that some previous findings on low molecular weight hepatic "receptors" for these polycyclic aromatic hydrocarbons may actually be explained in this way. Clearly, use of thoroughly perfused livers would seem to be a prerequisite for investigations on specific binding of aryl hydrocarbon hydroxylase inducers to liver cytosol. The rat hepatic TCDD receptor was also shown to be retained on DEAE-Sephacrose (eluted at 0.2-0.3 M NaCl), hydroxylapatite (eluted at 0.15-0.17 M phosphate), and heparin-Sepharose (eluted at 0.3-0.4 M NaCl). In conclusion, the TCDD receptor showed similar physicochemical and chromatographic characteristics to those previously reported for the androgen and glucocorticoid receptors. However, ligand competition experiments indicated that the TCDD receptor is not identical to any steroid receptor. In line with this, monoclonal anti-glucocorticoid receptor-IgG antibodies did not react with the TCDD receptor.

221. COMPETITIVE INTERACTION FOR THE AH RECEPTOR BY POLYCYCLIC AROMATIC HYDROCARBONS WHICH DO NOT INDUCE ARYL HYDROCARBON HYDROXYLASE IN C3H/10T1/2 MOUSE EMBRYO FIBROBLASTS

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Prog Clin Biol Res, 133:373-7 1983

Keywords (MeSH): Animal; Aryl Hydrocarbon Hydroxylase/*metabolism; Binding, Competitive; Carcinogens/*metabolism/pharmacodynamics; Cell Line; Fibroblasts/*metabolism; Mice; Mice, Inbred C3H; Polycyclic Hydrocarbons/*metabolism/pharmacodynamics; Receptors, Drug/*metabolism; Tetrachlorodibenzo-dioxin/metabolism.

222. THE AH REGULATORY GENE PRODUCT, SURVEY OF NINETEEN POLYCYCLIC AROMATIC COMPOUNDS AND FIFTEEN BENZO(A)PYRENE METABOLITES CAPACITY TO BIND TO THE CYTOSOLIC RECEPTOR

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Toxicol Lett, 10(1):109-110 1982

The capacity of 19 polycyclic aromatic compounds and 15 benzo(a)pyrene metabolites to displace [1,6-3H]-2,3,7,8-tetrachlorodibenzo-p-dioxin (3HTCDD) from a hepatic cytosol Ah receptor from C57BL/6N mice was measured by scintillation spectrometry, after removal of the unbound TCDD by dextran-coated charcoal and sucrose density gradient centrifugation of the remaining sample. TCDD, dibenzo(a,h)anthracene, dibenzo(a,c)anthracene, benzo(a)pyrene, and 3-methylcholanthrene were the most effective in displacement of 3HTCDD from the Ah receptor. There was good agreement between these results and previously published studies on induction of monooxygenase activities. The capacity of Hepa-1 cells to metabolize polycyclic aromatic compounds was approx (lowest to highest): TCDD less than benzo(e)pyrene less than benzo(a)anthracene less than 3-methylcholanthrene approx = dibenzo(a,h)anthracene, which has no relationship to the rankings of these compounds as displacers of 3HTCDD. For all 15 benzo(a)pyrene metabolites examined, there was generally a correlation between percent displacement of 3HTCDD from the Ah receptor and fold induction of aryl hydrocarbon hydroxylase (AHH) activity. For all the compounds tested there is a good correlation between the displacement of 3HTCDD from the Ah receptor and the capacity to induce AHH activity, also dye N-demethylase activity, or azoxamine metabolism. (36 Refs)

223. THE TCDD RECEPTOR IN RAT INTESTINAL MUCOSA AND ITS POSSIBLE DIETARY LIGANDS

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Nutr Cancer, 3(3):134-140 1982

Induction of aryl hydrocarbon hydroxylase (AHH) by polycyclic aromatic hydrocarbons and other inducers such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is known to occur following binding of the inducer to a soluble receptor protein similar to steroid hormone receptors. This receptor is usually called the TCDD receptor, since TCDD has the highest affinity of all known ligands for the receptor. In the present paper, a receptor for TCDD in cytosol from rat intestinal mucosa has been studied, using isoelectric focusing in polyacrylamide gel. This receptor's biochemical properties were found to be similar to those of the TCDD-receptor in rat liver cytosol. The dissociation constant (K_d) of the 3H-TCDD-receptor complex in rat intestinal mucosa was $0.7-3.1$ nM, and it was present at a concentration of 70-80 fmol/mg protein. Starvation did not significantly increase the receptor level. The affinities of some potential dietary ligands for the TCDD receptor in rat intestinal mucosa were also studied. Indole-3-carbinol had 1/2,600 of the affinity of TCDD for the receptor protein. Burylated hydroxyanisole (BHA), transstilbene oxide and quercetinpinemethylether competed even more weakly with 3H-TCDD for binding to the receptor. The biological significance of the occurrence of low-affinity ligands of dietary origin for the TCDD receptor is uncertain at the present time. (Author abstract) (37 Refs)

224. CYTOSOLIC AND NUCLEAR BINDING OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN TO THE AH RECEPTOR IN EXTRA-HEPATIC TISSUES OF RATS AND MICE

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Env J Biochem; 123(1):209-215 1982

Induction of aryl hydrocarbon hydroxylase (cytochrome P1-450) is mediated by the Ah receptor which initially binds inducing chemicals in the cytoplasm, after which the inducer-receptor complex translocates into the nucleus. Ah receptor can be detected and quantified by sucrose-density-gradient analysis of its binding to 2,3,7,8-tetrachloro[3H]dibenzo-p-dioxin. Labeling in vitro of cytosols from C57BL/6J mice and Sprague-Dawley rats reveals Ah receptor in several tissues, with concentrations in the rank order for C57BL/6J mice: liver greater than lung much greater than kidney approx = thymus ap prox = small intestine greater than prostate; for Sprague-Dawley rats: liver approx = lung approx = thymus much greater than small intestine much greater than greater than kidney. Cytosol receptor was not detectable by assays in vitro on skin, skeletal muscle, heart, brain or testis of C57BL/6J mice or Sprague-Dawley rats, nor was specific receptor detectable in cytosol from any tissue of genetically 'non-responsive' DBA/2J mice. Injection in vivo of 2,3,7,8-tetrachloro[3H]dibenzo-p-dioxin led to recovery of specific nuclear Ah receptor from liver, lung and kidney of both genetically 'responsive' C57BL/6J mice and 'non-responsive' DBA/2J mice as well as Sprague-Dawley rats. Nuclear Ah receptor concentrations were lower in DBA/2J mice than in C57BL/6J mice receiving the same dose, and nuclear receptor was detectable only with injection of high doses of 2,3,7,8-tetrachloro[3H]dibenzo-p-dioxin. Although cytosolic Ah receptor in DBA/2J mice appears to be 'defective' and cannot be detected directly by assays in vitro, recovery of specific receptor from nuclei of DBA/2J tissues implies that binding of 2,3,7,8-tetrachloro[3H]dibenzo-p-dioxin to Ah receptor does occur in vivo in these animals; this may explain the ability of 'non-responsive' DBA/2J mice to exhibit induction of aryl hydrocarbon hydroxylase when treated with the very potent inducer, 2,3,7,8-tetrachlorodibenzo-p-dioxin. (Author abstract) (12 Refs)

225. BINDING OF 3-METHYLCHOLANTHRENE AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN TO A COMMON AH RECEPTOR SITE IN MOUSE AND RAT HEPATIC CYTOSOLS

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The Ah receptor, which mediates induction of aryl hydrocarbon hydroxylase (cytochrome P1-450), previously was identified and characterized using 2,3,7,8-tetrachloro[3H]dibenzo-p-dioxin as the radioligand. Polycyclic aromatic hydrocarbons such as 3-methylcholanthrene induce the hydroxylase and compete with 2,3,7,8-tetrachloro[3H]dibenzo-p-dioxin for Ah receptor sites. 3-[3H]Methylcholanthrene is known to bind to several classes of cytosolic protein. Our main goal was to determine whether 3-[3H]methylcholanthrene and 2,3,7,8-tetrachloro[3H]dibenzo-p-dioxin bind to an identical receptor site, or whether each inducer might function by binding to its own unique receptor. Binding of the two radioligands in rodent hepatic cytosols was characterized using 2-h sucrose-density-gradient separations in a vertical-tube ultracentrifuge rotor. Overall evidence indicates that 3-

[3H]methylcholanthrene and 2,3,7,8-tetrachloro[3H]dibenzo-p-dioxin both induce aryl hydrocarbon hydroxylase by acting through the same receptor site. Although the two ligands have similar affinity for cytosolic Ah receptor sites, the relatively long biological half-life of the dioxin may account for its greater potency in vivo. (26 Refs)

226. CYTOSOLIC AND NUCLEAR BINDING PROTEINS FOR 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN THE RAT THYMUS

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Biochem Biophys Acta; 716(1):16-23 1982

The existence of a high-affinity, low-capacity 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-binding species was demonstrated in cytosol from rat thymus. Tests indicated that it was a protein. An excess of unlabeled 2,3,7,8-tetrachlorodibenzo-furan or beta-naphthoflavone displaced [3H]TCDD from the binder whereas phenobarbital, pregnenolone-16alpha-carbonitrile or dexamethasone did not compete. The apparent dissociation constant (Kd) of the [3H]TCDD-binder complex was determined to 0.36 nM and the apparent maximum amount of binding sites (Bmax) to 60 fmol/mg of cytosolic protein. When analyzed by sucrose density-gradient centrifugation at high ionic strength, the [3H]TCDD-binder complex sedimented at 4-5 S; at low ionic strength the complex sedimented more rapidly, probably due to aggregation. All these data support the interpretation that the demonstrated cytosolic TCDD-binder represents the receptor protein for TCDD. Following iv administration of [3H]TCDD, a low-capacity [3H]TCDD-macromolecule complex was extractable from thymic cell nuclei and was also identified as a protein. The nuclear [3H]TCDD-protein complex sedimented at 4-5 S at high ionic strength. Furthermore, a max uptake of [3H]TCDD in thymic nuclei was observed simultaneously with a decline in cytosolic radioactivity (at 3 h post-injection). These findings suggest that the nuclear [3H]TCDD-protein complex represented [3H]TCDD-receptor complex translocated from the cytoplasm. In conclusion, the rat thymus contains a cytosolic TCDD receptor at a concentration similar to that of the rat hepatic receptor. However, in vivo experiments showed that the nuclear uptake of [3H]TCDD (expressed as dpm/mg DNA) in the thymus was only about 6% of that in liver. Further studies are needed for an understanding of the mechanism behind this discrepancy. (28 Refs)

227. HIGH-AFFINITY BINDING OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN CELL NUCLEI FROM RAT LIVER

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Biochem Biophys Acta; 716(1):216-223 1982

The intranuclear binding of radioactive 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rat liver has been studied both in vivo and in vitro. Following the intravenous administration of [1,6-3H]TCDD, a maximum uptake by cell nuclei could be observed at 2 hr after injection with a concurrent decrease in the cytosolic uptake. Using linear sucrose density gradient centrifugation, dextran-coated charcoal adsorption assay, DEAE-Sepharose ion-exchange chromatography, competition, enzymatic and saturation studies, a high-affinity binding protein for TCDD in liver cell nuclei could be demonstrated both in vivo and after an exchange in vitro of intravenously administered unlabeled 2,3,7,8-tetrachlorodiben-

zofuran (TCDBF) for $[^3H]TCDD$. Sucrose density gradient analysis showed a size of 4-5 S for both the cytosolic and nuclear TCDD binding entity. The specific binding of $[^3H]TCDD$ to nuclear components was heat labile and saturable and had an equilibrium dissociation constant of 1.05 nM. Based on a differential susceptibility to specific hydrolyses, ie DNAase, RNAase, trypsin and pronase, the binding entity appears to be a 4-5 S salt-extractable protein. (Author abstract) (26 Refs)

228. REGULATORY GENE PRODUCT OF THE AH COMPLEX. COMPARISON OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND 3-METHYLCHOLANTHRENE BINDING TO SEVERAL MOIETIES IN MOUSE LIVER CYTOSOL

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J Biol Chem. 256(9):4584-4590, 1981

The major regulatory gene product of the murine Ah complex appears to be a cytosolic receptor. 2,3,7,8-[^3H]Tetrachlorodibenzo-p-dioxin or [^3H]3-methylcholanthrene binding to the Ah receptor and other moieties in hepatic cytosol was examined by gel permeation chromatography, velocity sedimentation (sucrose density gradient centrifugation), dextran-charcoal adsorption, and anion exchange chromatography. In the liver of Ah-responsive C57BL/6N and the Ahb/Ahb heterozygote, both radioligands bind to three major components: peak I, a large aggregate which is eluted in the void vol of Sephacryl S-300 columns and which sediments as a residue to the bottom of sucrose density gradients; peak II, an asymmetric protein (Mr approx = 245,000) with a Stokes radius of about 75 Å; and peak III, a globular protein (Mr approx = 87,000) with an estimated Stokes radius of 40 Å. The peak I aggregate is not adsorbed by dextran-coated charcoal and therefore represents the large proportion of nonsaturable radioligand binding measured by dextran-charcoal adsorption. The peak II protein has a size of about 9.0 S in low ionic strength and 7.5 S in high ionic strength, high affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and saturability at TCDD concentrations greater than about 1.0 nM. The peak II protein is not detectable in the liver of Ah-nonresponsive DBA/2N and the Ahb/Ahb homozygote and therefore represents the Ah receptor. The peak III protein has an estimated size of 5.0 S, is not saturable with either TCDD or 3-methylcholanthrene under the conditions of these experiments, and is not associated with the Ahb allele. 3-Methylcholanthrene binds to the peak III protein to a greater extent than TCDD. These data explain the discrepancies between the dextran-charcoal adsorption and sucrose density gradient assays. Any further studies of the function of the Ah receptor and these other ligand-binding moieties (eg nuclear translocation) should include gel permeation chromatography in order to distinguish among the various binding components. (Author abstract) (23 Refs)

229. TEMPERATURE-DEPENDENT CYTOSOL-TO-NUCLEUS TRANSLOCATION OF THE AH RECEPTOR FOR 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN CONTINUOUS CELL CULTURE LINES

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J Biol Chem. 256(23):11423-11427, 1981

The effective doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for inducing half-maximally (ED50 values) aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity (EC

1.14.14.1) in Hepa-1, H-4-II-E, HTC, and VERO continuous cell culture lines are about 0.45, 0.23, greater than 200, and 110 nM in the growth medium, respectively. The maximally induced hydroxylase activity is more than 100 x greater in 'responsive' TCDD-treated Hepa-1 and H-4-II-E than in 'nonresponsive' TCDD-treated HTC and VERO lines. The Ah receptor required for this induction process was assessed in these lines by sucrose density gradient analysis following dextran-charcoal treatment. With this assay we show (a) a temperature- and time-dependent cytosol-to-nucleus movement of the inducer-receptor complex in Hepa-1, H-4-II-E, and HTC cultures; (b) a TCDD concentration of about 0.28 nM required to saturate half of the nuclear specific binding sites in Hepa-1 cells; (c) of the total TCDD retained by cells in culture, an amount of less than 1% specifically bound to the receptor; (d) the lack of induction of receptor by benz[a]anthracene in the growth medium; (e) a greater thermostability of the receptor in the cytosol than the receptor in the nucleus; (f) sensitivity of both the cytoplasmic and nuclear receptor to proteolytic enzymes such as trypsin but not to nucleases; (g) the lack of detectable receptor in the nucleus of cells not exposed to TCDD in culture; and (h) a size of about 6 S for both the cytosolic and nuclear receptor, as estimated on sucrose density gradients in the presence of 0.4 M KCl. The nonresponsive VERO line has no detectable Ah receptor. The nonresponsive HTC line has about one-fourth as many receptors as the responsive Hepa-1 line (which has approx 5,000/cell) but has at least twice as many receptors as the responsive H-4-II-E cell line. The presence of the Ah receptor in the cytosol and its apparently normal translocation into the nucleus, such as in HTC cells, therefore does not guarantee that the induction process will proceed normally. (Author abstract) (30 Refs)

230. AN ESTIMATE OF THE MAXIMUM IN VIVO COVALENT BINDING OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN TO RAT LIVER PROTEIN, RIBOSOMAL RNA, AND DNA

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Cancer Res. 39(9):3341-3344, 1979

In light of the recently discovered potent carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in female rats, the in vivo covalent binding of [^3H]TCDD to rat liver macromolecules was reexamined. Immature Sprague-Dawley rats, receiving [^3H]TCDD (0.87 mCi/kg; specific activity, 39 Ci/millimole) concentrated 18%-64% of the total administered dose in their livers, but virtually all of this radioactivity (greater than 99.9%) was extractable. The max unextractable radioactivity was: protein, 60 picomoles (pmol) TCDD/mole (mol) of amino acid residue; ribosomal RNA, 12 pmol TCDD/mol of nucleotide residue; and DNA, 6 pmol TCDD/mol of nucleotide residue. If one assumes that this small residual amount of radioactivity represents covalent binding, this binding is four to six orders of magnitude lower than that of most chemical carcinogens, and the binding to DNA is equivalent to one molecule of TCDD per DNA, equivalent to 35 cells. The results suggest that it is unlikely that TCDD-induced oncogenesis is through a mechanism of covalent binding to DNA and somatic mutation. (29 Refs)

331. STUDIES ON THE MECHANISM OF THE CHLORINATED DIBENZO-P-DIOXINS AND RELATED COMPOUNDS (MEETING ABSTRACT)

Gronlund WF, Poland A

Univ. Rochester Sch. Medicine, Rochester, NY

The Scientific Basis for the Public Control of Environmental Health Hazards, held by the New York Academy of Sciences in New York, 21-30 June, 1978. The New York Academy of Sciences, New York, New York, 1978.

Halogenated dibenzo-dioxins such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and dibenzofurans, aro- and ary-benzenes, and biphenyls which are approx stereoisomers of TCDD, are potent inducers of the microsomal monooxygenase activity, arylhydrocarbon hydroxylase (AHH). We have isolated a cytosolic protein from liver which has the in vitro binding properties expected of the receptor for induction of AHH activity, ie the binding affinity of chlorinated aromatic hydrocarbon congeners corresponds to their potency to induce this enzyme activity. The TCDD-cytosolic binding protein complex translocates to the nucleus. Genetic evidence suggests that the receptor-ligand complex initiates not only the induction of AHH activity, but the expression of a number of structural genes. For congeners of each of the classes of halogenated aromatic compounds listed above there is an excellent correlation between their binding affinity for the cytosolic protein and their toxic potency. We propose that the initial event in the toxicity of these compounds is their binding to and sustained occupation of the cytosolic receptor. (no Refs)

B.

REGULATION OF GENE EXPRESSION

332. MUTATIONS AFFECTING THE REGULATION OF TRANSCRIPTION OF THE CYTOCHROME P1-450 GENE IN THE MOUSE HEPA-1 CELL LINE

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J Biol Chem; 258(1):1790-5 1983

The cytochrome P1-450-dependent activity, aryl hydrocarbon hydroxylase, and cytochrome P1-450 messenger RNA levels were studied in wild-type Hepa1c7 cells and in aryl hydrocarbon hydroxylase-deficient mutants derived from this line. Tetrachlorodibenzo-p-dioxin (TCDD) induced both parameters approximately 50-fold in Hepa1c7 cells. Mutants in genes B and C that are affected in the functioning of the Ah receptor required for hydroxylase induction and dominant mutants had nondetectable or much reduced P1-450 mRNA levels and hydroxylase activities after TCDD treatment. Hybrids between wild-type cells and the dominant mutants were also deficient in these parameters. The dominant mutants therefore appear to express a trans-acting repressor of cytochrome P1-450 transcription. Mutants in gene A were heterozygous. Some lacked the mRNA completely; others were inducible for it; while still others (subgroup IV) had high levels even when they were grown without TCDD. These results suggest strongly that gene A is the structural gene for cytochrome P1-450. When subgroup IV mutants were cultured together with wild-type cells, they failed to express P1-450 mRNA in the absence of TCDD treatment. These cells probably accumulate an inducer that can be metabolized by aryl hydrocarbon hydroxylase. The inducer did not appear to be a component of the medium and therefore may be an endogenous ligand of the Ah receptor.

333. ANALYSIS OF AH GENE LOCUS BY SOMATIC CELL HYBRIDIZATION: EXPRESSION OF AN REGULATORY GENE PRODUCT FOR 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN MOUSE L-CELL X MOUSE HEPATOMA CELL HYBRIDS

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Department of Biology, University of Windsor, Windsor, Ontario, Canada.
Somatic Cell Mol Genet; 11(1):53-61 1985

Properties of the aryl hydrocarbon hydroxylase (AHH) enzyme system were examined in polycyclic aromatic hydrocarbon (PAH) -noninducible L-cell x PAH-inducible hepatoma (Hepa) mouse cell hybrids. In hybrids, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces AHH activity. The levels of maximal TCDD-induced AHH activity in the hybrids and the Hepa parent are similar, although a greater concentration of TCDD is required for expression in the hybrids. This concentration difference appears to reflect dilution of AHH-associated gene products by the L-cell parent rather than altered gene expression. The regulatory gene product, the Ah receptor, is expressed similarly in the hybrids and Hepa parent. Both demonstrate specific, high-affinity binding of [³H]TCDD to an equivalent number of receptor sites per cell. These results suggest that the molecular mechanism of phenotypic resemblance to the inducible Hepa parent (i.e., 'dominance') in the mouse L-cell x Hepa hybrids involves expression of only the Hepa Ah gene complex.

334. DEVELOPMENTAL AND TISSUE-SPECIFIC DIFFERENTIAL REGULATION OF THE MOUSE DIOXIN-INDUCIBLE P1-450 AND P3-450 GENES

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Dev Biol; 112(1):177-84 1985

The murine Ah locus has two structural genes, P1-450 and P3-450, that are members of the 2,3,7,8-tetrachlorodibenzo-p-dioxin-inducible P-450 gene family and are closely linked on mouse chromosomes 9. Transcriptional activation of both genes in liver is controlled by the Ah receptor. Inducible P1-450 mRNA is correlated with aryl hydrocarbon hydroxylase activity, whereas inducible P3-450 mRNA is correlated with acetanilide 4-hydroxylase activity. This report shows that inducible P3-450 mRNA is also highly correlated with estradiol 2-hydroxylase activity. Both P1-450 and P3-450 activities are induced by 3-methylcholanthrene in liver; in contrast, P1-450 but not P3-450 activity is induced to a significant extent in kidney, lung, and intestine. Constitutive levels of P3-450 mRNA in liver are at least five times greater than those of P1-450 mRNA, thus accounting for the much greater 'fold inducibility' of aryl hydrocarbon hydroxylase than acetanilide 4-hydroxylase or estradiol 2-hydroxylase. The hepatic P3-450 induction response occurs at lower inducer concentrations than the P1-450 induction response, suggesting a difference in affinity of the inducer-receptor complex for regulatory regions of the two genes. Developmentally, P1-450 inducibility occurs at least 2 weeks earlier in gestation than P3-450 inducibility, which occurs near the time of birth. These data thus demonstrate striking differences between the expression of two homologous genes in the same P-450 subfamily, with respect to developmental and tissue specificity and sensitivity to common inducers that interact with the Ah receptor.

235. AUTOREGULATION PLUS UPSTREAM POSITIVE AND NEGATIVE CONTROL REGIONS ASSOCIATED WITH TRANSCRIPTIONAL ACTIVATION OF THE MOUSE P1(450) GENE

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Nucleic Acids Res; 13(20):7269-80 1985

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is known to interact with a cytosolic receptor and, in turn, activate transcription of the mouse P1(450) gene. Various lengths of DNA upstream of the P1(450) gene were inserted into the pSV0-cat expression vector, with and without addition of the Harvey murine sarcoma virus (Ha-MSV) 72-bp repeat enhancer element. The constructs were cotransfected with pSV 2-neo into mouse hepatoma wild-type cells and two variant cell lines. One variant is believed to result from a mutation in the P1(450) structural gene and expresses high levels of P1(450) mRNA constitutively; the other variant has a defect in nuclear translocation of the inducer-receptor complex. After selection in G418, the cells were treated with control medium, TCDD, cycloheximide, or TCDD plus cycloheximide and then assayed for chloramphenicol acetyltransferase (CAT) activity. The data are consistent with the presence of several functional regions within the upstream sequence: a promoter region, a region that is negatively autoregulated, possible repressor-binding and inducer-receptor complex-binding sites, and an upstream activation element that is required for transcriptional activation by TCDD. The Ha-MSV enhancer can substitute for this upstream activation element.

236. SUPERINDUCTION OF CYTOCHROME P1-450 GENE TRANSCRIPTION BY INHIBITION OF PROTEIN SYNTHESIS IN WILD TYPE AND VARIANT MOUSE HEPATOMA CELLS

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J Biol Chem; 260(9):5648-55 1985

Inhibition of protein synthesis superinduces transcription of the cytochrome P1-450 gene in Hepa 1c1c7 mouse hepatoma cells. The superinduced transcription rate is 10-15-fold higher than the maximal rate of transcription induced by the known inducer 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alone. Superinduction is maximal within 30-40 min and remains maximal for at least 90 min. Cytochrome P1-450 mRNA is the same length in TCDD-induced and superinduced cells. Superinduction does not occur in variant cells in which TCDD-receptor complexes bind weakly to nuclei and which do not transcribe the cytochrome P1-450 gene in response to TCDD. Inhibition of protein synthesis does not alter several properties of TCDD-receptor complexes. The results imply that a second control mechanism modulates the action of the TCDD-receptor complex in regulating cytochrome P1-450 gene transcription.

237. CONTROL OF CYTOCHROME P1-450 GENE EXPRESSION BY DIOXIN

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Science; 227(4693):1439-502 1985

The environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may produce its effects by altering gene expression in susceptible cells. In mouse hepatoma cells, TCDD induces the transcription of the cytochrome P1-450 gene, whose product, aryl hydrocarbon hydroxylase, contributes both to the detoxification and to the metabolic activation of carcinogenic polycyclic aromatic hydrocarbons. A

DNA fragment containing sequences flanking the 5' end of the cytochrome P1-450 gene was isolated and analyzed. This DNA fragment contains a cis-acting control element with at least three functional domains: a putative promoter, an inhibitory domain upstream from the promoter that blocks its function, and a TCDD-responsive domain still farther (1265 to 1535 base pairs) upstream of the promoter. These findings, together with results from earlier studies, imply that transcription of the cytochrome P1-450 gene is under both positive and negative control by at least two trans-acting regulatory factors.

238. THE DIOXIN-INDUCIBLE P-450 GENE FAMILY IN THE MOUSE AND HUMAN (MEETING ABSTRACT)

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Third International Symposium on Biological Reaction International, June 6-8, 1985, College Park, MD, abstract 5, 1985

By sequence analysis and/or chromosomal mapping, it is presently clear that a minimum of five gene families exist in the P-450 gene superfamily: phenobarbital-inducible; TCDD-inducible; steroid-inducible; the P-450_{ac} important for cholesterol side-chain cleavage; and the steroid C-21 polymorphism. This laboratory has concentrated its efforts on the TCDD-inducible P-450 gene family because of its important role in chemical carcinogenesis and toxicity. The TCDD-inducible P-450 gene family has two members in mouse, only one in man. We have isolated and sequenced the genes and flanking regions for mouse P1-450 (benzo(a)pyrene metabolism), mouse P3-450 (acetanilide metabolism), and human P1-450. Full-length cDNA clones were sequenced first, in order to determine unequivocally each exon-intron junction. With the eukaryotic expression vectors pSV0-neo and pSV0-cat and DNA upstream from the promoter regions of the three genes, four regions of DNA are defined as essential for the induction response: (a) a major enhancer element epistatic to a (b) minor enhancer element; (c) a TCDD-Ah receptor binding region; and (d) a repressor(s)-binding site. In all three genes, three highly homologous regions (11-12 bp) upstream between -280 and -550 from the cap site are noted as candidates for DNA-binding regulatory proteins. It can be shown that the TCDD-inducible P-450 gene family diverged (without any evidence of inversion or rearrangement) from the phenobarbital-inducible P-450 gene family 200-1000 million yr ago (MYA) and that P1-450 (probably via gene duplication) split from P3-450 about 65 MYA. The mouse P1-450 and P3-450 genes have been mapped near Mpi-1 (middle of chromosome 9); the human P1-450 gene has been localized to chromosome 15. By use of a human 5' cDNA probe, induced P1-450 mRNA is highly correlated with induced aryl hydrocarbon (benzo(a)pyrene) hydroxylase activity in human mitogen-activated lymphocyte cultures treated with 3-methylcholanthrene. Although restriction fragment length polymorphisms (RFLPs) in the human P1-450 gene are readily detected, no clear-cut relationship between RFLP pattern and P1-450 mRNA inducibility has yet been found. Hence, although a rger screening of RFLPs in the human population and family studies are indicated, it is possible that genetic differences in human cancer risk associated with the Ah locus will be found in the regulatory (Ah receptor) gene(s) rather than the P1-450 structural gene.

239. THE MURINE AH LOCUS. COMPARISON OF THE COMPLETE CYTOCHROME P1-450 AND P3-450 CDNA NUCLEOTIDE AND AMINO ACID SEQUENCES

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J Biol Chem. 259(17):10705-13 1984

Mouse cytochrome P1-450 and P3-450 are most closely associated with induced aryl hydrocarbon (benzo[a]pyrene) hydroxylase (EC 1.14.14.1) and acetanilide 4-hydroxylase activity, respectively. Full-length cDNA clones of P1-450 and P3-450 were generated from mRNA isolated from 3-methylcholanthrene-treated C57BL/6N mouse liver. P1-450 cDNA is 2620 nucleotides in length and has a coding region (base 110 to 1,675) that produces a protein with 521 residues (Mr = 58,914). P3-450 cDNA is 1,894 nucleotides in length and yields a protein with 513 residues (Mr = 58,185). P1-450 mRNA is the first reported example in mouse in which UAG is used as the termination codon. P1-450 and P3-450, both induced by polycyclic hydrocarbons and regulated by the Ah receptor, exhibit overall nucleotide and protein homology of 68, and 73%, respectively. Segments of high homology, interspersed with regions of low homology, support the hypothesis of gene conversion or unequal crossing over as possible mechanisms for divergence of these two genes. Mouse P1-450 and P3-450 cDNAs were compared with previously published data on rat P-450c cDNA and rabbit form 2 protein, corresponding to two P-450 genes from the 'phenobarbital inducible' P-450 gene subfamily. Nucleotide homology between a member of either gene subfamily is about 30%, and protein homology is about 15%, suggesting that the Ah locus-associated P-450 gene subfamily diverged from the phenobarbital inducible P-450 subfamily more than 200 million years ago. An N-terminal and a C-terminal cysteinyl fragment corresponding to the regions around P1-450 Cys-150 and Cys-458, respectively, are the only two cysteinyl peptides conserved among all four proteins compared. Because of greater homology in the C-terminal conserved cysteinyl fragment between the two gene subfamilies and a greater hydrophobic pocket in the C-terminal conserved cysteinyl fragment, the data favor this cysteine as the more likely candidate for the thiolate ligand to the heme iron in the P-450 enzyme active-site.

240. REGULATION OF MOUSE CYTOCHROME P3-450 BY THE AH RECEPTOR. STUDIES WITH A P3-450 CDNA CLONE

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Biochemistry. 23(25):6005-8 1984

The Ah locus in the C57BL/6N mouse regulates at least two cytochrome P-450 gene products, termed in the mouse P1-450 and P3-450; these two enzymes are so named because each is responsible for the highest turnover number for the substrates benzo[a]pyrene and acetanilide, respectively. A cDNA library was prepared in pBR322 from sucrose gradient fractionated total liver poly(A⁺)-enriched RNA (approximately 20 S) from 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) treated C57BL/6N (Ahh/Ahh) mice. Differential colony hybridization screening, with [³²P]cDNA probes derived from total liver mRNA of both TCDD-treated and control C57BL/6N mice, yielded pP(3)450-21 (1710 base pair) and pP(1)450-57 (1770 base pair) cDNA clones. pP(1)450-57 was found to have 690 base pairs 5'-ward of the original P1-450 cDNA cloned in this laboratory. Restriction maps of pP(3)450-21 and pP(1)450-57 are markedly different and clearly are derived from separate genes. By means of hybridization-translation-arrest experiments, anti-(P3-450) precipitates the translation product (Mr approximately equal to 55000) of mRNA specifically hybridizing to pP(3)450-21. It is also shown that hybridization-translation-arrest experiments using polyclonal antibodies are not specific for proof of a P-450 cDNA clone. pP(3)450-21 was used to probe liver mRNA from Ahb/Ahb, Ahb/Ahd, and Ahd/Ahd mice treated with 3-methylcholanthrene, beta-naphthoflavone, aroclor 1254, isosafrole, low TCDD, or high TCDD. These genetic data rigorously demonstrate control of the P3-450 (20S) mRNA induction process by the Ah receptor. pP(3)450-21 fragments hybridized to TCDD-induced C57BL/6N mRNA and to a portion of the cloned 5' end of the P1-450 gene from a mouse MOPC 41 plasmacytoma library. (ABSTRACT TRUNCATED AT 250 WORDS)

241. 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN RECEPTORS IN WILD TYPE AND VARIANT MOUSE HEPATOMA CELLS. NUCLEAR LOCATION AND STRENGTH OF NUCLEAR BINDING

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J Biol Chem. 259(21):980-5 1984

The volume of aqueous solvent present during subcellular fractionation of mouse hepatoma (Hepa 1c1c7) cells influences the distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) receptors between the nuclear and cytosolic fractions. When the effects of dilution are minimized, at least 90% of the receptors associate with nuclei. The receptors bind relatively strongly to nuclei, as measured by their release by KCl. TCDD-receptor complexes bind more strongly to nuclei than do unoccupied receptors. A temperature-dependent event further enhances the binding of TCDD-receptor complexes to nuclei. A class of variant cells contains receptors which bind relatively weakly to nuclei; this defect accounts for the variant phenotype. We conclude that, in the intact cell, TCDD receptors are located within the nucleus and that the temperature-dependent event in the induction of cytochrome P1-450 gene expression is one which strengthens the binding of the TCDD-receptor complex to chromatin.

242. CLONING GENES THAT ENCODE ENZYMES WHICH METABOLIZE DRUGS AND CHEMICAL CARCINOGENS

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Biochemical Basis of Chemical Carcinogenesis. Gross H, Jung R, Kramer M, Marquardt H, Oesch F, eds. New York: Raven Press, p. 89-105, 1984

The Ah locus governs the induction of numerous drug-metabolizing enzymes and other proteins by polycyclic aromatic compounds such as 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), cytosolic Ah receptor is the gene product of the Ah regulatory gene. It has been proposed that these polycyclic aromatic inducers compete with epidermal growth factor (EGF) for the cell-surface receptor in the same order as that seen for compounds competing with TCDD for the cytosolic Ah receptor. The inducers also cause epidermal keratinization, birth defects, immunosuppression, and 'promoter' effects of chemical carcinogenesis. These and other considerations suggest that the Ah locus may be involved in certain growth processes such as differentiation and cancer promotion in addition to the previously discovered phenomenon of induction of drug-metabolizing enzymes. Studies on the cloning of genes that encode enzymes which metabolize drugs and chemical carcinogens are

described under the following section headings: problems with the P-450 proteins, characterization of antibody to P1-450, fundamental principles of recombinant DNA technology and isolation of a P1-450 cDNA clone, potential pitfalls in 'translation arrest' experiments for genes encoding membrane-bound proteins, genetic proof for the P1-450 cDNA clone, and isolation of the P1-450 genomic gene. As a result of the study, it was proposed that it may be possible to develop an assay, based on recombinant DNA technology, for assessing the human Ah phenotype; such an assay may predict who is at increased risk for certain types of environmentally caused cancers. (39 Refs)

243. BIOCHEMICAL AND GENETIC ANALYSIS OF VARIANT MOUSE HEPATOMA CELLS WHICH OVERTRANSCRIBE THE CYTOCHROME P1-450 GENE IN RESPONSE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

Jones PB, Miller AG, Israel DI, Galaszi DR, Whitlock JP Jr
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J Biol Chem. 259(20):12557-63 1984

Using the fluorescence-activated cell sorter, we have isolated a population of variant mouse hepatoma cells which have a markedly increased ability to metabolize benzo(a)pyrene. Compared with wild-type (Hepa 1c1c7) cells, the variant cells exhibit increased aryl hydrocarbon hydroxylase activity and increased responsiveness of the aryl hydrocarbon hydroxylase induction mechanism to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Cell fusion experiments indicate that the variant phenotype is co-dominant with respect to wild-type. Filter hybridization analyses indicate that increased accumulation of cytochrome P1-450-specific mRNA accounts for the overproduction of aryl hydrocarbon hydroxylase activity. Measurements of RNA synthesis in isolated nuclei reveal that the variants exhibit an increased rate of transcription of the cytochrome P1-450 gene in response to TCDD. The variant cells contain no detectable alteration in their TCDD receptors, nor is the cytochrome P1-450 gene amplified in the variants. Filter hybridization analyses of restriction endonuclease-digested DNA indicate that the variant cytochrome P1-450 gene is relatively undermethylated, compared with the wild-type gene. We conclude that the variant cells contain an altered cis-acting genomic element(s) which regulates the expression of the cytochrome P1-450 gene.

244. STRUCTURAL GENE PRODUCTS OF THE AH LOCUS. TRANSCRIPTIONAL REGULATION OF CYTOCHROME P1-450 AND P3-450 mRNA LEVELS BY 3-METHYLCHOLANTHRENE

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Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, Bethesda, Maryland
Mol Pharmacol. 26(1):117-21 1984

Mouse liver cytochromes P1-450 and P3-450 represent those forms of polycyclic hydrocarbon-induced P-450 most closely associated with induced aryl hydrocarbon (benzo(a)pyrene) hydroxylase and acetanilide 4-hydroxylase activity, respectively. These two proteins are controlled by the Ah receptor. C57BL/6N mice possess the high-affinity receptor; DBA/2N mice, the poor-affinity receptor. 3-Methylcholanthrene at the highest dose technically possible induces both proteins in C57BL/6N but not DBA/2N mice, whereas sufficiently high doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induce both proteins in both inbred mouse strains. Plasmids containing DNA complementary to P1-450 and P3-450 mRNA, respectively, were used in an *in vitro* nuclear transcription assay to determine the mechanism of the induction response. In C57BL/6N mice, transcriptional rates of the P1-450 and

P3-450 genes increase dramatically as early as 3 hr after 3-methylcholanthrene treatment and at 12 hr reach maximal levels of 20- and 15-fold, respectively, above control values. In contrast, no increase in either gene is found in 3-methylcholanthrene-treated DBA/2N mice. Following TCDD administration, both P1-450 and P3-450 gene transcription rates are elevated in DBA/2N mice. There is a 3- to 6-hr lag period between the early onset of enhanced transcription rates and the later rise in P1-450 and P3-450 mRNA. Basal and induced levels of P3-450 mRNA are about 5-fold greater than those of P1-450 mRNA. These data confirm that the 3-methylcholanthrene and TCDD induction responses, governed by the Ah receptor, are mediated principally through an increase in specific gene transcription.

245. REGULATION OF CYTOCHROME P1-450 GENE TRANSCRIPTION BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN WILD TYPE AND VARIANT MOUSE HEPATOMA CELLS

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J Biol Chem. 259(9):5400-2 1984

Using RNA synthesized in isolated nuclei, we have measured the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the rate of synthesis of cytochrome P1-450 mRNA in wild type mouse hepatoma (Hepa 1c1c7) cells and in variant (BPr1) cells, which fail to accumulate the TCDD-receptor complex within the nucleus. In wild type cells, TCDD induces a 20-fold increase in the rate of synthesis of cytochrome P1-450 mRNA within 30 min. This effect persists for at least 18 h. In contrast, TCDD has no effect on cytochrome P1-450 mRNA synthesis in the variant cells. The results demonstrate that TCDD increases the rate of transcription of the cytochrome P1-450 gene and suggest that transcription requires nuclear localization of the TCDD-receptor complex.

246. TCDD INCREASES AND CYCLOHEXIMIDE FURTHER ENHANCES THE RATE OF TRANSCRIPTION OF THE CYTOCHROME P-450 GENE (MEETING ABSTRACT)

Israel DI, Whitlock JP Jr
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DNA 3(1):100 1984

We have measured the rate of synthesis of cytochrome P1-450 mRNA in nuclei isolated from wild-type (Hepa 1c1c7) and variant mouse hepatoma cells. Nascent RNA was elongated *in vitro* in the presence of [³²P]UTP and was hybridized to excess cytochrome P1-450 cDNA (clone 46 [Negishi et al., PNAS 78:800, 1981]) immobilized on nitrocellulose filters. In wild-type cells, 1 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) produces about a 25-fold increase in the rate of synthesis of cytochrome P1-450 mRNA. This increase is half-maximal 20 minutes after exposure to TCDD. The maximal rate of mRNA synthesis occurs approximately 30 minutes after TCDD administration and is maintained for at least 18 hours. In BPr C1 cells, a variant cell line which fails to localize the TCDD-receptor complex to the nucleus (Miller et al., JBC 258:3525, 1983), TCDD has no effect on cytochrome P1-450 gene transcription. If protein synthesis is inhibited by cycloheximide (10 ug/ml) during exposure to TCDD, the maximal rate of synthesis of cytochrome P1-450 mRNA is about 10-fold greater than the maximal rate in the presence of TCDD alone. This 'superinduction' is half-maximal 15 minutes and maximal 30 minutes after addition of cycloheximide. In the absence of TCDD, cycloheximide has little effect on the rate of synthesis of cytochrome P1-450 mRNA. Neither TCDD nor cycloheximide increases the rate of tran-

scription of the CAD gene or Lys-actin gene, indicating that the inducing effects of TCDD and cycloheximide may be specific for cytochrome P1-450 mRNA. These results indicate that TCDD induces the accumulation of both cytochrome P1-450 mRNA and aryl hydrocarbon hydroxylase activity by increasing the rate of synthesis of cytochrome P1-450 mRNA. This increase in the rate of transcription requires nuclear localization of the TCDD-receptor complex. Enhancement of the rate of synthesis by cycloheximide implies that another regulatory factor(s), in addition to the TCDD-receptor complex, controls the expression of the cytochrome P1-450 gene.

347. INDUCTION OF mRNA SPECIFIC FOR CYTOCHROME P1-450 IN WILD TYPE AND VARIANT MOUSE HEPATOMA CELLS

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J Biol Chem. 258(17):10390-4 1983

We have used a cDNA probe specific for an aromatic hydrocarbon-inducible form of cytochrome P-450 to analyze the accumulation of enzyme-specific mRNA in wild type Hepa 1c1c7 cells and in variant cells defective in aryl hydrocarbon hydroxylase induction. In wild type cells, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) produces a 25-50-fold increase in the steady state concentration of cytochrome P1-450-specific RNA. This RNA binds to oligo(dT)-cellulose and migrates in agarose gels as a single species of about 2900 nucleotides. Dot hybridization analyses of total RNA indicate that half-maximal induction of P1-450 mRNA occurs at about 10 pM TCDD; maximal induction occurs at about 100 pM TCDD. Parallel increases occur in aryl hydrocarbon hydroxylase activity. RNA induction precedes aryl hydrocarbon hydroxylase induction by 2-4 h. Kinetic analyses of mRNA induction in response to submaximal concentrations of TCDD reveal no change in the degradation of P1-450 mRNA during induction. We detect an increase in P1-450 mRNA 30 min after TCDD administration. Full accumulation of mRNA occurs in cells in which protein synthesis is inhibited by 95-97%. Variant cells with decreased TCDD receptors exhibit decreased basal and inducible levels of P1-450 mRNA. Variant cells with a defect in nuclear localization of the inducer-receptor complex exhibit virtually no basal or inducible levels of P1-450 mRNA. Hybrid cells formed by fusing these different variants contain wild type basal and inducible levels of P1-450 mRNA. We conclude that expression of the cytochrome P1-450 gene is under transcriptional control and requires nuclear localization of the TCDD receptor.

348. BIOCHEMICAL AND GENETIC ANALYSIS OF VARIANT MOUSE HEPATOMA CELLS DEFECTIVE IN THE INDUCTION OF BENZO(a)PYRENE-METABOLIZING ENZYME ACTIVITY

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J Biol Chem. 258(6):3523-3527 1983

We have analyzed wild type mouse hepatoma (Hepa 1c1c7) cells and variant cells which are defective in the induction of benzo(a)pyrene-metabolizing enzyme activity. One type of variant has no detectable basal or inducible aryl hydrocarbon hydroxylase activity. This class contains apparently normal cytosolic receptors for 2,3,7,8-tetrachlorodibenzo-p-dioxin, but is unable to translocate the inducer-receptor complex to the nucleus. The second type of variant has low levels of basal and inducible aryl hydrocarbon hydroxylase activity. This class contains cytosolic receptors which are decreased either in their number or in their ability to bind 2,3,7,8-tetrachlorodibenzo-p-dioxin; translocation of the inducer-receptor complex to the nucleus is apparently normal. Cell fusions

indicate that both variant phenotypes are recessive with respect to wild type. Complementation analyses indicate that the defects are located on different genes. (Author abstract) (39 Refs)

349. REGULATORY GENE PRODUCT OF THE AH LOCUS. CHARACTERIZATION OF RECEPTOR MUTANTS AMONG MOUSE HEPATOMA CLONES

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J Biol Chem. 257(11):6402-6407 1982

The major regulatory gene product of the Ah locus is a cytosolic receptor which binds avidly to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Ah receptor levels in the cytosol and accumulation of the inducer-receptor complex in the nucleus have been examined in the mouse hepatoma cell culture parent line Hepa-1c1c7 and in six mutant clones that exhibit varying degrees of deficiency in aryl hydrocarbon (benzo(a)pyrene) hydroxylase (EC 1.14.14.1) inducibility. One of these clones (c3) is dominant, whereas the others are known to be recessive and to belong to three distinct complementation groups (and, therefore, reflect mutations in three different genes): c1 and c5, c2 and c6, and c4. Clones c1, c3, and c5 have essentially normal Ah receptor levels, compared with the wild type Hepa-1c1c7 parent, possess normal kinetics for translocation of the inducer-receptor complex into the nucleus, and yet exhibit very low or non-detectable basal or inducible aryl hydrocarbon hydroxylase activity; these clones could represent a mutation in the P1-450 structural gene or other genes responsible for the induced hydroxylase activity. Clones c2 and c6 are receptor-deficient mutants, having no more than 10% of wild type Ah receptor levels, normal kinetics of nuclear translocation of the inducer-receptor complex, and no more than 20% of wild type hydroxylase inducibility by either TCDD or benzo(a)anthracene. The defect in c2 cells was shown by both in vitro and in vivo TCDD-binding studies not to reflect a lack of receptor saturability. Clone c4 has normal cytosolic levels of Ah receptor, is defective in nuclear translocation of the inducer-receptor complex, and lacks any detectable basal or inducible aryl hydrocarbon hydroxylase activity. (Author abstract) (35 Refs)

350. THE AH LOCUS: CORRELATION OF INTRANUCLEAR APPEARANCE OF INDUCER-RECEPTOR COMPLEX WITH INDUCTION OF CYTOCHROME P1-450 mRNA

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Cell. 31(1):275-284 1982

The Ah locus regulates the induction of cytochrome P1-450 by foreign chemicals such as 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin. The induction process is controlled by the cytosolic Ah receptor. The cytosolic and nuclear Ah receptor were studied in the liver from inbred C57BL/6N (Ahh/Ahh) mice, inbred DBA/2N (Ahd/Ahd) mice and heterozygotes (Ahh/Ahd) and homozygotes (Ahd/Ahd) derived from the (C57BL/6N x DBA/2N)F1 x DBA/2N backcross. After [3H]-1,6[2,3,7,8-tetrachlorodibenzo-p-dioxin (3H-TCDD) is given in vivo, the receptor in Ahh/Ahh and Ahh/Ahd mice is detectable in the cytoplasm and nucleus; in Ahd/Ahd mice the receptor is not measurable in the cytosol, but is found in the nucleus at levels one fourth to one fifth of those in Ahh/Ahh mice. P1-450 (23S) mRNA content was estimated by Northern hybridization and by R10 analysis with a mouse P1-450 cloned cDNA. An excellent

dose-response relationship ($r = 0.99$) was found between the amount of 3H-TCDD-Ah receptor complex appearing in the nucleus and the quantity of P1-450 mRNA induced in mice with all three possible Ah genotypes. (Author abstract) (48 Refs.)

251. ISOLATION AND CHARACTERIZATION OF A CLONED DNA SEQUENCE ASSOCIATED WITH THE MURINE AH LOCUS AND A 3-METHYLCHOLANTHRENE-INDUCED FORM OF CYTOCHROME P-450

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Proc Natl Acad Sci USA, 78(2):part 2:800-804, 1981

Using partially purified mouse liver 25S mRNA known to be associated with the Ah locus and 3-methylcholanthrene-induced cytochrome P1-450, we synthesized double-stranded cDNA by the successive action of reverse transcriptase (RNA-directed DNA nucleosideyltransferase) and the Klenow A fragment of *Escherichia coli* DNA polymerase I. The double-stranded cDNA was inserted into pBR322 plasmid DNA by Pst I cleavage and homopolymeric 'tailing' and cloned in *E. coli* LE392. Clone 46 hybridized with [32P]cDNA made from 25S mRNA from 'Ah-responsive' C57BL/6N mice but did not hybridize with similarly prepared [32P]cDNA from 'Ah-unresponsive' DBA/2N mice. Clone 30 was positive, and clone 7 was negative, with both C57BL/6N and DBA/2N [32P]cDNA probes; these two clones were therefore used as 'positive' and 'negative' control clones, respectively. By translation-arrest experiments, clone 46 DNA and clone 30 DNA were shown to be associated with anti-P1-450 and anti-albumin-precipitable material, respectively. By agarose gel electrophoresis of Pst I digests, the clone 46 DNA insert was shown to be 1,100 base pairs in total length and to contain one internal Pst I site. The cDNA made from total mRNA isolated from 3-methylcholanthrene-treated C57BL/6N mice hybridized to the two fragments of Pst I-digested DNA from clone 46, whereas similarly prepared cDNA from 3-methylcholanthrene-treated DBA/2N and control C57BL/6N and DBA/2N mice did not. Of 11 restriction endonucleases used, two (Pst I and Xba I) had sites within the clone 46 DNA insert. After hybridization of clone 46 32P-labeled nick-translated DNA to EcoRI fragments from A/Hel mouse genomic DNA and fractionation by RPC-5 chromatography and gel electrophoresis, only one positive band (3.4 kilobase pairs) appeared. These data demonstrate conclusively that pBR322 clone 46 DNA is associated with mRNA controlled by the murine Ah locus, presumably the structural gene encoding 3-methylcholanthrene-induced P1-450. (Author abstract) (34 Refs)

C.

REGULATION OF TOXIC RESPONSES

252. REGULATION OF EPIDERMAL GROWTH FACTOR BINDING IN A HUMAN KERATINOCYTE CELL LINE BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Toxicol Appl Pharmacol, 77(2):251-9, 1985

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) decreased the binding of epidermal growth factor (EGF) by the human keratinocyte cell line SCC-12F. This response was concentration dependent (half-maximal effective concentration, $EC_{50} = 1.8$ nM) and stereospecific. Scatchard analysis of EGF binding indicated that treatment with TCDD resulted in a loss of high-affinity ($K_d = 0.28$ nM) binding sites. This loss

was accompanied by a concomitant inhibition of EGF-stimulated DNA synthesis. The kinetics for the decrease of EGF binding by TCDD and benzo(a)pyrene (BP) were compared. Inhibition of EGF binding by BP was maximal by 24 hr, with 90% recovery of EGF binding apparent by 48 hr. In contrast, TCDD treatment for 72 hr was required to produce maximal inhibition, and no recovery was evident up to 10 day after removal of TCDD from the growth medium. The data indicate that modulation of EGF binding by TCDD was mediated by the Ah receptor. Subsequent cellular responses, for example, inhibition of EGF-stimulated DNA synthesis, may be important in the expression of altered differentiation patterns observed in human epidermal keratinocytes exposed to TCDD.

253. THE ROLE OF RECEPTORS IN 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) TOXICITY

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Department of Toxicology, Uppsala University, Sweden
Arch Toxicol Suppl, 8:43-60, 1985

There is good evidence that the Ah-(TCDD-) receptor plays a role in the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and its congeners. TCDD and other chlorinated aromatic hydrocarbons with chlorine atoms in lateral positions (2,3,7,8-tetrachlorodibenzofuran, 3,3',4,4'-tetrachlorodibenzene, 3,3',4,4'(5,5')-tetra(hexachlorophenyl), all bind to the receptor and show a similar pattern of toxicity, although there is a wide range in potency. The Ah-receptor is viewed as the major product of the regulatory gene of the Ah-locus in the mouse. Several of the toxicities of TCDD and congeners (teratogenesis, thymic involution and hepatic porphyria) have been shown to segregate with the Ah-locus. In vitro studies using keratinizing cells or fetal thymus organ culture have shown a good correlation between activity as ligands of the receptor and toxicity for the compounds discussed. The great differences in toxic potency of these compounds in vivo may therefore be a result of variation in rate of metabolism and excretion rather than differences in affinity for the Ah-receptor. The physiological role of the Ah-receptor is discussed, whether it has developed as a response to exposure to toxic substances in the environment, as a means of induction of P-450-dependent polysubstrate mono-oxygenase activities in order to make those substances more liable for excretion - or is there a physiological ligand? TCDD has a long half-life in the body, and a sustained competition for binding to the receptor between TCDD and a ligand of importance for normal cell functions may result in toxicities such as the wasting syndrome. This tentative ligand could be of varying importance in different species, which might explain the great variation in sensitivity between species, the hamster being about 5000 times less sensitive than the guinea pig. (64 Refs)

254. SENSITIVITY TO SUPPRESSION OF CYTOTOXIC T CELL GENERATION BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IS DEPENDENT ON THE AH GENOTYPE OF THE MURINE HOST

Nagarkatti PS, Sweeney GD, Gaudin J, Clark DA
Toxicol Appl Pharmacol, 72(1):169-176, 1984

Susceptibility of mice to a variety of toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is genetically determined by the Ah locus. To determine if immunotoxicity following TCDD exposure was also regulated by the Ah locus, we tested the ability of low dose TCDD (4 ng/kg) to suppress the generation of alloreactive cytotoxic T cells (CTL) by lymphocytes from 'susceptible' C57BL/6, and 'resistant'

DBA/2, and from C57BL/6XDBA/2J F1 hybrid mice *in vitro*. To determine in TCDD acts directly on the 'susceptible' lymphoid cells, the immune response of C57BL/6 leads to DBA/2 and DBA leads to C57BL/6 bone marrow chimeras was also measured. C57BL/6 and F1 mice proved susceptible to suppression consistent with the dominant effect of Ah. Susceptibility to suppression in chimeric mice, however, was determined by the Ah genotype of the host and not by the genotype of the grafted lymphomyeloid cells. Mixing experiments demonstrated that suppression of CTL generation by TCDD was due to suppressor T cells. The frequency of CTL precursors was not affected by TCDD. These results are consistent with the idea that TCDD acts by an Ah locus-dependent mechanism to indirectly promote development of suppressor T cells that block the generation of CTL from their precursors.

295. EFFECTS OF CYTOCHROME P1-450 INDUCERS ON THE CELL-SURFACE RECEPTORS FOR EPIDERMAL GROWTH FACTOR, PHORBOL 12,13-DIBUTYRATE, OR INSULIN OF CULTURED MOUSE HEPATOMA CELLS

Karencamp SO, Eisen HJ, Henderson O, Nisbet DW
Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, Bethesda, Maryland
J Biol Chem. 258(17):10578-83 1983

Hepa-1c1c7, a mouse hepatoma cell line, was used to study the effect of cytochrome P1-450 inducers on the binding of 125I-epidermal growth factor (EGF), 125I-insulin, or [20-3H]phorbol 12,13-dibutyrate each to its specific cell-surface receptor. After a 24-h exposure to the cultured cells, several polycyclic hydrocarbon P1-450 inducers decrease the binding of EGF to EGF receptors much more than phenobarbital does. There appears to be a selectivity in the inhibitory effects: whereas EGF binding to EGF receptors is blocked, the binding of either phorbol ester or insulin each to its specific cell-surface receptors remains unaffected. The rank order of binding affinities of these chemicals to the cytosolic Ah receptor (2,3,7,8-tetrachlorodibenzo-p-dioxin) much greater than benzo[a]pyrene greater than benzo[a]anthracene greater than 6-aminochrysene much greater than phenobarbital is not correlated with their effects on EGF binding capacity. The effect of polycyclic hydrocarbons on EGF binding takes 24 h at 37 degrees C to be maximal, whereas phorbol 12-myristate 13-acetate, a potent tumor-promoting compound, inhibits EGF binding in less than 30 min. Removal of benzo[a]anthracene from the growth medium after 24 h results in a gradual recovery in EGF binding, indicating that the effect is reversible. Benzo[a]pyrene and benzo[a]anthracene are relatively ineffective at decreasing EGF binding to the EGF receptors in Hepa-1 mutant clones c2 and c4, which lack a normally functioning Ah receptor and inducible aryl hydrocarbon hydroxylase activity (P1-450). The very toxic metabolite (+)-7 beta,8 alpha-dihydroxy-9 alpha, 10 alpha-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, when added directly to the growth medium of c4 cells, however, is effective at decreasing EGF binding. These data suggest that electrophilic metabolites of polycyclic aromatic compounds, formed by P1-450 induced during the exposure of Hepa-1 cells to these chemicals, are important in decreasing EGF binding to the EGF cell-surface receptor. Occupancy of the Ah receptor per se does not affect EGF binding.

296. RESPONSE OF MURINE EPIDERMIS TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN: INTERACTION OF THE AH AND HR LOCI

Knutton JC, Poland A
Cell. 99(1):225-234 1982

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related halogenated aromatic hydrocarbons produce epidermal hyperplasia, hyperkeratosis and sebaceous gland metaplasia in the skin of mice bearing the recessive mutation (hr/hr) hairless. This response is mediated through the cytosol receptor protein: the structure-activity relationship for receptor binding corresponds to that for production of skin lesion, and these histopathological changes segregate with the generic polymorphism at the Ah locus, the locus determining the cytosol receptor. In HRS/J mice, an inbred strain segregating for the hr locus, both hairless (hr/hr) and haired (hr/+) mice possess the high-affinity cytosol receptor and respond to TCDD with the induction of epidermal aryl hydrocarbon hydroxylase activity, a receptor-mediated biochemical response; however, only hr/hr mice develop the proliferative/metaplastic skin response. We propose a genetic model for the interaction of the Ah and hr loci, to account for the differential response to TCDD observed in the skin of HRS/J hr/hr and hr/+ mice.

297. 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN. SEGREGATION OF TOXICITY WITH THE AH LOCUS

Poland A, Glover E
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Mol Pharmacol. 17(1):86-94 1980

Induction of thymic atrophy and teratogenesis by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and the segregation of these toxic effects with the Ah locus were studied. Ah locus controls the receptor that mediates induction of aryl hydrocarbon hydroxylase (AHH) and other enzymes coordinately expressed. The mice were C57BL/6J (C3) (high affinity receptor and sensitivity to AHH induction), DBA/2J (DB) (low affinity receptor and less sensitivity to AHH induction), and (B6D2F1/J) (B6) mice, hybrids derived from these two strains. C3 were more sensitive to thymic atrophy induced by single injection of TCDD than were DB; hybrid mice showed intermediate sensitivity. To see if this toxic effect of TCDD segregated with the Ah locus, B6 (heterozygous, Aa, for the locus) were mated with DB (homozygous, aa) and the offspring were phenotyped; heterozygous (Aa) offspring were more sensitive to TCDD than were homozygous (aa) offspring. TCDD congeners or polycyclic aromatic hydrocarbons with high affinities for the receptor produced thymic atrophy in C3, while congeners and PAH with low affinities did not. Measurements of specific binding of 3H-TCDD in cytosol of liver and thymus from C3 and DB showed high affinity saturable binding of TCDD in both tissues from C3 and virtually absent binding in DB tissues. C3 also had higher rates of uptake of TCDD into liver than DB. TCDD induced large increases in monooxygenase activity in liver of C3 but only slight increases in this enzyme activity in thymus of C3. Teratogenic effects of TCDD were assessed by determining the incidence of cleft palates in fetuses exposed to TCDD. In 5/5 strains with low receptor affinity, TCDD produced a low incidence of cleft palates; in 4/5 strains with high receptor affinity, TCDD produced high incidences of cleft palate. (30 Refs)

VI.

ENVIRONMENTAL OCCURRENCE,
PUBLIC HEALTH ISSUES, AND
REGULATIONS CONCERNING DIOXINS
AND DIBENZOFURANS

A.

ENVIRONMENTAL OCCURRENCE

258. THE ENVIRONMENTAL FATE OF COMBUSTION-
GENERATED POLYCHLORINATED DIBENZO-P-
DIOXINS AND DIBENZOFURANS

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Dis Abstr Int JBL 46(1):103-4 (1985)

Polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) are formed in various combustion processes. Because of the extreme toxicity of certain PCDD and PCDF isomers, it is important to understand the sources and long-term environmental fate of dioxins and dibenzofurans. Our goal is to address three questions: (a) Are the PCDD and PCDF found in the environment from combustion or industrial sources? (b) Are PCDD and PCDF stable, and thus accumulating in the environment? (c) Is there some evidence regarding the specific combustion processes (trash, chemical waste, coal or wood combustion) which are the major sources of dioxins and dibenzofurans? We measured the PCDD and PCDF in coal and municipal incinerator fly ash, using capillary gas chromatography, negative chemical ionization, and mass spectrometry. We found approximately equal concentrations of the tetra- to octachlorodibenzo-p-dioxins and tetra- to heptachlorodibenzofurans in combustion-derived particulates. We analyzed urban air particulates from St. Louis, Missouri and Washington, DC. Unlike the combustion samples, we found a predominance of octachlorodibenzo-p-dioxin (OCDD). The predominance of OCDD in these samples suggests that this isomer is the most stable in the environment. We analyzed sediments from the Great Lakes, Siskiwit Lake (Isle Royale), and lakes in Switzerland. The isomer profiles were remarkably similar to those found in air particulates, which indicated that the atmosphere, and thus, combustion was the source of PCDD and PCDF in these sediments. Dated sediment cores were analyzed to determine the historical input of dioxins and dibenzofurans to the environment. In sediment cores, PCDD and PCDF concentrations increased at the sediment depth corresponding to 1940; before 1940, no significant amounts of PCDD and PCDF were detected. This 1940 horizon correlates with trends for the production of chlorinated aromatic compounds by the chemical industry. The PCDD and PCDF horizon cannot be due to coal combustion, wood combustion, or forest fires. Thus, the PCDD and PCDF presently accumulating in the environment are the result of the combustion of wastes containing chlorinated precursors of dioxins and dibenzofurans. (Author abstract)

259. THE LIVER AND ENVIRONMENTAL POISONS

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Klin Wochenschr 63(22):1193-5 (1985)

For almost a century now numerous examples of acute and subacute hepatic injury from exposure to toxic agents in the occupational or non-occupational environment have been extensively studied and are well documented, but such events are comparatively rare. In contrast, epidemiological data associating exposure to environmental chemicals with chronic

liver disease or primary hepatic malignancies in the human is scarce as compared with the vast body of literature concerning chronic pulmonary disease as a consequence of exposure at the workplace. Large-scale industrial production of many newly synthesized organic chemicals began during the period 1930-1940 but it was not until the 1960s that the output increased exponentially. Consequently, the spectrum of environmental influences is gaining increasing complexity since simultaneous or sequential exposure to a variety of pollutants is becoming the rule rather than the exception. Possible interaction or synergism of environmental agents - even of those which in themselves or for their low dosage level may be considered 'harmless' - and particularly latency periods of more than one decade further complicate preventive strategies. The liver, as the central site for the biotransformation of xenobiotics, deserves special attention when new chemicals which are to be introduced into the environment are being tested for their potential toxicity, especially since many hepatotoxic agents have been shown to undergo bioactivation in the liver. Currently available information on hepatic injury due to environmental agents is briefly reviewed and comprises solvents and degreasing agents, pesticides, polychlorinated biphenyls, dioxins and dibenzofurans, epoxy resin hardeners, vinyl chloride, naturally occurring hepatotoxins in plants and fungi, herbal medicines, and traditional remedies and a side-light on the Reye syndrome and the Spanish 'toxic oil syndrome'.

260. SCIENTIFIC PANEL: HARMFUL SUBSTANCES IN
BREAST MILK

Anonymous

Arch Gynecol 239(1-4):99-216 (1985)

Keywords (MeSH): Benzofurans/analysis; Body Burden; Dioxins/analysis; Drugs/*analysis; Female; Human; Hydrocarbons; Chlorinated/analysis; Metals/analysis; Milk; Human/*analysis; Mycotoxins/analysis; Nitrates/analysis; Nitrites/analysis; Nitrosamines/analysis; Polycyclic Hydrocarbons/analysis; Pregnancy; Risk.

261. DIOXINS IN THE ENVIRONMENT

Roland WF

Schweizer Ver Wasser Boden Lufthyg 65 403-12 (1985)

Keywords (MeSH): Animal; Benzofurans/toxicity; Carcinogens; Comparative Study; Dioxins/*toxicity; Environmental Monitoring; Environmental Pollutant/*toxicity; Isomerism; Lethal Dose 50; Tetrachlorodibenzodioxin/analogs and derivatives/toxicity.

262. FORMATION OF POLYCHLORINATED
DIBENZOFURANS AND DIOXINS DURING
COMBUSTION, ELECTRICAL EQUIPMENT FIRES
AND PCB INCINERATION

Hutzinger O, Choudhry GG, Jüttner BG, Johnston LE

Environ Health Perspect 63:1-9 (1985)

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are likely formed by thermal synthesis of a variety of primary precursors. Highest levels of these compounds are expected, however, when the starting material requires only one or two reaction steps for their formation, as is the case with chlorophenols, chlorobenzenes and polychlorinated biphenyls (PCBs). Laboratory pyrolyses have indeed shown that PCBs give significant yields of PCDFs, and chlorobenzenes give both PCDFs and PCDDs. In addition, a variety of other chloroaromatic compounds are formed. From these experiments and from accidents involving PCB fires, it is known that PCDFs are the most important toxic compounds associated with PCBs. Most commercial PCBs contain PCDFs in the low ppm range. PCDF con-

centration does not increase during normal operation in electrical equipment. Accidents (fires and explosions) involving PCBs can give PCDF levels of soot of up to 1000 ppm and higher. Effective thermal destruction of PCB is possible in modern incineration units, provided high temperatures, excess air and sufficient residence times are used. Exact figures for minimum temperature and residence time cannot be given, since feedstock and incinerator construction greatly influence destruction efficiency. Effluents from EPA-licensed incinerators used for PCB destruction contain only very low levels of PCDDs and PCDFs.

263. PCB-CONTAINING TRANSFORMER FIRES: DECONTAMINATION GUIDELINES BASED ON HEALTH CONSIDERATIONS

Milby TH, Miller TL, Forester TL
J Occup Med. 27(5):551-6 (1985)

Realization that polychlorinated biphenyl (PCB)-containing (askarel) transformer fires can produce toxic polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzodioxin (PCDD) isomers has led to efforts to develop guidelines for cleanup of structures contaminated by soot from these fires. Two such fires have occurred in the United States in recent years, one in 1981 in Binghamton, N.Y., the other in 1985 in San Francisco. The phenomenon of PCB transformation to more toxic substances under conditions of uncontrolled combustion was studied in both locations, but much more extensively in New York. In terms of possible danger to human health, PCDFs comprise by far the most important PCB combustion products identified in the soot of both fires. PCDDs were not formed in sufficient quantity in either fire to constitute an important health hazard. The PCDF isomers of greatest health significance are the tetra-, penta-, and hexa- forms having all four lateral positions (2,3,7 and 8) chlorinated. Separate decontamination guidelines were established for each fire, based on different toxicity and exposure assumptions. One guideline was based on carcinogenic risk assessment of 2,3,7,8-TCDD and the other on experimental animal no-observed-effect level data for the same compound. More effort will be required to develop a generally accepted set of askarel fire decontamination guidelines.

264. WASTE DISPOSAL TECHNOLOGIES FOR POLYCHLORINATED BIPHENYLS

Peyer WT, Lindstrom FT
Environ Health Perspect. 59:163-177 (1985)

Improper practices in the disposal of polychlorinated biphenyl (PCB) wastes by land burial, chemical means and incineration distribute these chemicals and related compounds such as polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) throughout the environment. The complete range of methods for disposal that have been proposed and are in use are examined and analyzed, with emphasis given to the two most commonly used methods: land burial and incineration. The understanding of aquifer contamination caused by migration of PCBs from subsurface burial sites requires a description of the physical, chemical and biological processes governing transport in unsaturated and saturated soils. For this purpose, a model is developed and solved for different soil conditions and external driving functions. The model couples together the fundamental transport phenomena for heat, mass, and moisture flow within the soil. To rehabilitate a contaminated aquifer, contaminated groundwaters are withdrawn through drainage wells, PCBs are extracted with solvents or activated carbon and treated by chemical, photochemical or thermal methods. The chemical and photochemical methods are reviewed, but primary emphasis is devoted to the use of incineration as the

preferred method of disposal. After discussing the formation of PCDFs and PCDDs during combustion from chloroaromatic, chloroaliphatic, as well as organic and inorganic chloride precursors, performance characteristics of different thermal destructors are presented and analyzed. To understand how this information can be used, basic design equations are developed from governing heat and mass balances that can be applied to the construction of incinerators capable of more than 99.99% destruction with minimal to nondetectable levels of PCDFs and PCDDs.

265. AGRICULTURALLY-RELATED CARCINOGENIC RISK

Berzsony M, Tornik G, Pinar A, Sarson A
National Institute of Hygiene, Budapest, Hungary
IARC Sci Publ. 156:403-60 (1984)

Although all people are exposed throughout life to pesticides (during manufacture and use, in the environment, via the food chain), with the exception of vinyl chloride and the arsenic compounds, no human carcinogen pesticide is known. In experimental animals and short-term tests, however, many pesticides have proved to be carcinogenic or genotoxic. In order to elucidate possible modes of action related to genotoxicity (short-term tests) and carcinogenicity, data on 83 pesticides were analysed. A definitive promoter effect was demonstrated for five compounds, and a suspected promoter effect could be attributed to 16 compounds. Almost all were organohalogenes. Ethylenebithiourea and aminotriazole are also carcinogens, which induce tumours through hormonal imbalance. Ethylenebithiourea is the degradation product and metabolite of certain ethylene-bis(dithiocarbamate) pesticides. Chlorophenol and chlorophenoxyacetic acid-type pesticides contain 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a contaminant. TCDD has definite promoter action. Seventeen tumorigenic pesticides could be regarded as genotoxic, and similar activity could be suspected in three other carcinogenic pesticides. Many pesticides contain N-nitroso impurities in various amounts. Nitrosation of the original pesticide or its derivative, such as dialkylamines, alkyl-arylamines, methylenamines, give rise to the formation of N-nitroso compounds. Amides containing secondary amines, such as ethylcarbamate and alkylurea, could be nitrosated in vivo and in vitro. Most nitrosated pesticides are strong genotoxic carcinogens.

266. 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

World Health Organization
Geneva, Switzerland
Environ Health Criteria. 29:1-15 (1984)

The health risks of exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) are discussed under the following headings: summary and recommendations for further studies; properties and analytical methods; sources of environmental pollution; environmental transport, distribution, and transformation of 2,4-D; environmental levels and exposure; chemobio-kinetics and metabolism; effects of 2,4-D on animals; effects on man: clinical and epidemiological studies; and evaluation of health risks to man from exposure to 2,4-D. Most of the 2,4-D residues result from the production and use of 2,4-D herbicides. Another possible minor source of 2,4-D is the use of 2,4-dichlorophenoxybutyric acid. Various amounts of 2,4-D products applied to a target area may be distributed in the general environment within a few hours or days by the movements of air, water or soil, particularly during periods of rain, high winds, or high temperature. 2,4-D and its derivatives are broken down fairly rapidly by hydrolysis, photolysis and biological action. Nothing is known about the environmental fate of the impurities present in 2,4-D herbicides. Exposure to 2,4-D, in the work environment of per-

sons producing, handling, or using herbicides may result in absorption of detectable amounts of 2,4-D. The carcinogenic potential of 2,4-D in animals has not been adequately tested. Scientifically valid studies have not shown any adverse reproduction effects in human beings accidentally or occupationally exposed to 2,4-D. The results of studies suggesting that occupational exposure to 2,4-D may result in chromosome abnormalities are equivocal. The results of some epidemiological studies have suggested an association between exposure to phenox herbicides and increased incidence of malignant tumors and tumor mortality. It is not clear, at present, whether this represents a true association, and if so, whether it is specifically related to 2,4-D. Recommendations for further studies are made. In the case of occupationally-exposed workers, further consideration should be given to the chemokinetics of 2,4-D under conditions of repeated exposure.

265. INCIDENCE AND LEVELS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN LAKE ONTARIO

Ryan JJ, Law P, Pilon JC, Lewis D, McLeod HA, Gervais A
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Environ Sci Technol. 18(9):719-21 1984

The extent and frequency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in commercial fish from Lake Ontario were studied because of the interest in TCDD as a pollutant of fish and other wildlife. Fish were chosen from Lake Ontario, as it has a substantial commercial fishery while showing some of the highest organochlorine contamination of all the Great Lakes. Sixty-two samples of fish comprising 10 different species were analyzed by using a validated extraction, cleanup, and detection procedure. TCDD was found in 21 samples with values ranging from 2.0-38.5 parts per trillion (ppt). Twelve of the samples had TCDD levels above 10 ppt and 5 were above 20 ppt. Seven of the 10 species of commercial fish had detectable levels of TCDD. The TCDD values for different fish species appear to fall into three groups. The sunfish family, rock bass, pumpkinseed and black crappie, had no detectable levels of TCDD; white perch, yellow perch, brown bullhead, and white sucker were found to contain intermediate levels below 10 ppt; channel catfish, American eel and rainbow smelt had values almost always greater than 1.0 ppm. PCB values appeared to parallel the fat content in most cases, as fish with low or high PCB content also had low or high PCB content also had low or high lipid levels. Three fish species showed high levels of chlorinated hydrocarbon contamination. The presence of high levels of PCB (greater than 1 ppm) and a high lipid content (greater than 3%) indicated a high probability of dioxin contamination. Comparison of this data with those generated by other laboratories from all the Great Lakes indicate that larger species of commercial fish such as channel catfish and carp, particularly from Lake Ontario and part of Lake Huron have the highest levels and incidence of TCDD.

268. 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN SEDIMENT SAMPLES FROM LOVE CANAL STORM SEWERS AND CREEKS

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Dept. Health, Albany, NY, 12201
Environ Sci Technol. 17(1):6-10 1983

New York State Department of Health and Department of Environmental Conservation undertook a study to determine the nature and extent of contamination of Love Canal area. Concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) from 0.9-312 nanog/g were determined in six storm

sewer and creek sediment samples. The extent of human exposure resulting from the presence of TCDD in the Love Canal area is unknown. (18 Refs)

269. EXPOSURE TO POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZO-FURANS IN THE ENVIRONMENT

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Department of Oncology, University Hospital, Umeå, Sweden
Prog Clin Biol Res. 132E:357-69 1983

Keywords (MeSH): Animal; Benzo(a)pyrene/*adverse effects/analysis; Carcinogens/analysis; Cardiac; Dioxins/*adverse effects/analysis; Environmental Exposure; Female; Fishes; Human; Male; Meat/analysis; Mice; Neoplasms/*chemically induced; Polychlorobiphenyl Compounds/*adverse effects/analysis; Poultry.

270. IMPLICATIONS OF TREATING WATER CONTAINING POLYNUCLEAR AROMATIC HYDROCARBONS WITH CHLORINE: A GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC STUDY

Otley AR, Luskhonen RJ, Lohavongjits M, Cox LM, Puder DA, Carlson RM
Dept. Chemistry, Univ. Minnesota, Duluth, MN, 55812
Environ Health Perspect. 46:75-86 1982

The products of aqueous chlorination reactions of 1-methylnaphthalene, fluorene, dibenzofuran, anthracene, phenanthrene, 1-methylphenanthrene, fluoranthene, and pyrene have been determined. The conditions employed for these reactions approximated those that might be encountered in water treatment facilities. Reactions at pH greater than 6 tended to produce oxygenated products (epoxides, phenols, quinones, etc.), and reactions at pH less than 6 tended to produce both oxygenated (quinones) and chlorinated products. (Author abstract) (47 Refs)

271. WATER, PESTICIDES IN GROUND WATER BENEATH IRRIGATED FARMLAND IN NEBRASKA, AUGUST 1978

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Conservation Survey Div., Inst. Agriculture Natural Resources,
Univ. Nebraska, Lincoln, NE
Proc. Monit. J. 14(2):70-73 1980

During the 1978 irrigation season, 14 ground water samples were collected in an area where nitrate-nitrogen (NO₃-N) concentrations exceeded 10.0 mg/liter. The 13 pesticides analyzed included residues of atrazine, 2,4-D, and simazine and were present in concentrations well below the limits given in the interim primary drinking water standards. Although the low levels of atrazine present in the ground water are not toxic to humans, atrazine and high amounts of NO₃-N in the ground water may form N-nitrosatrazine in nitrogen-fertilized soils. (17 Refs)

272. ENVIRONMENTAL POLLUTION AND PREGNANCY: RISKS AND UNCERTAINTIES FOR THE FETUS AND INFANT

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Am J Obstet Gynecol. 137(2):162-175 1980

Numerous environmental contaminants can affect the developing embryo, fetus or infant. This essay explores such questions as these: What is the importance in mutagenesis and teratogenesis of macroenvironmental pollutants such as the heavy metals, dioxin derivatives, polychlorinated biphenyl compounds, and pesticides? What is the significance of microenvironmental pollutants (or social environmental factors)

such as tobacco smoke, alcohol, and pharmacologic agents over which exposed individuals have considered control? What are some of the ethical and legal implications of these toxins of which clinicians should be aware? (Author abstract) (100 Refs)

273. CHEMICAL DISEASE IN HUMANS: PROBLEMS IN COMPARATIVE TOXICOLOGY

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Ciba Found Symp. 76:331-347 1980

Since the latent periods for human tumors induced by such chemicals as vinyl chloride, Thorotrast, beta-naphthylamine, benzidine, and asbestos may be 20-30 yr, the carcinogenic potentials of more recently introduced xenobiotics (eg, polychlorinated biphenyls, polybrominated biphenyls, or dioxin) cannot yet be determined. Xenobiotics may persist and accumulate in tissue stores. Some chemicals may seemingly disappear from the body, but leave a tissue imprint of their effects that appears decades after exposure ceases. Estimates of biologically active doses are sometimes complicated by the presence of highly toxic trace contaminants and homologs of varying biologic activities. The effects of chemical exposure on the tissues may be obscured by the absence of the characteristic symptoms normally associated with the disease. The lack of coherence between laboratory results and the clinical effects of enzyme-inducing agents may be explained by interactions between two or more xenobiotics or between the chemical(s) and genetic or metabolic variables. Since there is much evidence that biologic processes are strikingly similar in different mammalian species, the toxicologic significance to humans of enzyme induction and related processes requires careful evaluation. (22 Refs)

274. FOREST PESTICIDES: AN OVERVIEW

Josephson J

No affiliation given

Environ Sci Technol. 14(10):1165-1168 1980

The controversy over the use of forest pesticides, particularly the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), is discussed. In addition, the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a contaminant of 2,4,5-T, are considered. 2,4-D is of special interest since it may be carcinogenic and teratogenic; in addition, it apparently causes other adverse effects in humans. Cancer and other adverse health effects in persons exposed to the defoliant Agent Orange (a combination of 2,4-D and 2,4,5-T) could be caused by TCDD. Rats exposed to 0.1 µg/kg (2,200 parts per trillion) TCDD daily for 2 yr showed an increased incidence of hepatocellular and squamous-cell carcinomas of the lung, hard palate, nasal turbinates, and tongue. (no Refs)

275. DIOXIN: THE LINGERING CONTROVERSY

Cressland J

No affiliation given

Ecologist. 10(1):87-95 1980

The controversy concerning the use of dioxin, a contaminant of the popular herbicide 2,4,5-T (found in Agent Orange), is discussed with respect to whether dioxin accumulates in the environment and whether symptoms of ill health reported by veterans of the Vietnam War resulted from dioxin exposure. The production and use of 2,4,5-T is reviewed. New experiments have shown dioxin to be carcinogenic in animals, even at very low doses. Human exposure to 2,4,5-T in the United States and in Vietnam is discussed. In a model ecosystem, it was shown that dioxin accumulated in the food chain. However, actual evidence for the accumulation of dioxin in the

environment has been difficult to collect. Dioxin has been found in cattle fed on land that had been sprayed with 2,4,5-T, and in breast milk of women living in areas that had been sprayed. Legislation banning the use of 2,4,5-T in certain areas has been passed, but it is still used on rangelands and for rice growing. Further studies are needed. (29 Refs)

276. 2,3,7,8-TETRACHLORODIBENZOFURAN AS A COMMERCIALLY AVAILABLE 99% PURE POLYCHLORINATED BIPHENYL ISOMER IDENTIFIED AS THE INDUCER OF HEPATIC CYTOCHROME P-448 AND ARL HYDROCARBON HYDROXYLASE IN THE RAT

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Drug Metab Dispos. 6(3):238-264 1978

Four contaminants, 2,3,7,8-tetrachlorodibenzo-furan (TCDF), 2,3,8-trichlorodibenzo-furan, 2,3,7-trichlorodibenzo-p-dioxin, and 1,2,4,7,8-pentachlorodibenzo-p-dioxin, were identified in commercially available 99% pure 2,4,5,2',4',5'-hexachlorobiphenyl (HC9). TCDF was the major contaminant (44 ppm). There were less than 2 ppm of each of the other contaminants. The presence of these contaminants dramatically influenced the hepatic effects of this isomer in the rat. Rigorously purified 2,4,5,2',4',5'-HCB resembled the phenobarbital class of inducers of drug-metabolizing enzymes. In contrast, the contaminated polychlorinated biphenyl (PCB) isomer possessed the inducing properties of both the barbiturate and 3-methylcholanthrene classes of inducers. The major contaminant, TCDF, was a 3-methylcholanthrene-type inducer. Its potency was sufficient to account for the mixed-type of induction seen with the commercial lot of 2,4,5,2',4',5'-HCB. The induction of cytochrome P-448 and aryl hydrocarbon hydroxylase was due to TCDF. The induction of cytochrome P-450 and N-demethylase was due to the PCB isomer. Previous studies with relatively pure isomers may have attributed effects to PCB's that actually resulted from contamination by extremely low levels of dibenzofurans. The identification of dibenzofurans and dibenzo-o-dioxins in a 99% pure PCB isomer reveals a serious problem, inasmuch as many chlorinated environmental chemicals contain these contaminants, and because historically, 99% purity has been considered sufficient for use in pharmacological and toxicological studies. (Author abstract) (22 Refs)

277. IDENTIFICATION OF POLYCHLORINATED DIBENZOFURAN ISOMERS IN FLY ASH AND PCB PYROLYSES

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Chemosphere. 7(5):419-429 1978

The polychlorinated dibenzofurans (PCDF) content of two samples of fly ash was determined. The fly ash samples were obtained from an industrial heating facility and a municipal incinerator. Gas chromatography-mass spectroscopy showed that the heating facility fly ash contained 0.3 ppm PCDFs while the incinerator fly ash contained 0.1 ppm. In addition, these samples showed approx the same level of polychlorinated biphenyls (PCBs). Mass fragmentograms showed the elution of tri- to hepta-CDFs in both fly ash samples and remarkable similarities in the pattern of the PCDF isomers. This indicated a common source for these compounds. Similar patterns of isomers were also observed in pyrolyses of Aroclor 1254 and Aroclor 1260. The results support the assumption that the PCDFs in the fly ash were, to a significant extent, due to the uncontrolled burning of PCBs. The most

toxic isomers were the major constituents in fly ash as well as in the PCB pyrolysates. Careful control of the disposal of PCBs by incineration is recommended in order to minimize environmental and occupational exposure to hazardous PCDFs. (14 Refs)

B.

ASSAY SYSTEMS FOR DIOXINS AND RELATED COMPOUNDS

278. STRUCTURE-ACTIVITY RELATIONSHIPS OF THE POLYHALOGENATED AROMATIC HYDROCARBONS

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Dist Abstr Int JBL 45(12):1807-68 (1983)

The H-4-II E cell enzyme induction and Ah receptor bioassays were utilized to examine the biochemical potencies of environmental samples, and the structure-activity relationships of polyhalogenated aromatic hydrocarbons (PAHs). Rank order comparisons between the two bioassays with environmental samples were comparable, although the H-4-II E cells were found to be a more sensitive and reliable indicator of potential in vivo toxicity, particularly with those samples having little biochemical activity. The H-4-II E cell enzyme induction EC50 values of environmentally significant reconstructed polychlorinated biphenyl (PCB) and dibenzofuran (PCDF) mixtures were found to be an additive response to the enzyme inducing potencies of their constituents. Structure-activity studies with PCDFs demonstrated that the most toxic congeners in vivo were also the most potent AHH and EROD inducers (in vivo and in vitro), and bound with the highest affinity to the cytosolic receptor. The in vitro data also suggested that chloro substitution of PCDFs at C-4 and C-6 may result in congeners which are more active than isomers with C-1 or C-9 chloro groups. Structure-activity studies with a series of chlorinated and brominated tetrahalobiphenyls demonstrated that the biologic activity of these compounds was enhanced with increasing bromine substitution. Quantitative structure-activity relationship analysis of four series of PAHs was carried out. For the 7-substituted 2,3-dichlorodibenzo-p-dioxins and the 8-substituted 2,3-di- and 2,3,4-trichlorodibenzofurans, only the lipophilicity of the lateral substituent influenced the relative binding affinities. This was in sharp contrast to the 4'-substituted 2,3,4,5-tetrachlorobiphenyls, where the lipophilic, electronic and hydrogen bonding parameters of the 4'-substituent determined the binding constants. In all four series, the enzyme induction was not found to be a linear function of binding. With the exception of the dibenzo-p-dioxins, EROD and AHH induction were linearly related. The relationship between EROD and AHH induction by the 7-substituted 2,3-dichlorodibenzo-p-dioxins was dependent on the lipophilicity of the substituent. Enzyme induction studies with White Leghorn cockerels using classical enzyme inducers, and selected PCB congeners and commercial mixtures suggested that the coordinate enzyme induction and toxicity responses noted in rats was not observed in the avian due to a unique mixed-function oxidase enzyme induction response. (Author abstract)

279. PLANT TISSUE CULTURE AS A MODEL SYSTEM FOR MUTAGENICITY TESTING OF CHEMICALS

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Mutat Res 147(1-2):51-7 (1985)

Two karyotypically stabilized callus strains of *Crepis capillaris* ($2n = 7$ and $2n$ greater than or equal to 12, respectively) were employed to illustrate the utility of plant tissue culture method for screening and analysis of cytogenetic effects of

chemicals following long-term treatment. The cytogenetic analysis of callus cells revealed significant differences in the toxic and mutagenic effects of chemicals under study (2,4-D, MH, NMU and kinerin). A certain dose-response relationship (though not necessarily linear) was observed. The maximum cytogenetic activity of chemicals was associated with certain intermediate concentrations (4×10^{-5} M- 9×10^{-5} M), whereas a further increase in the dose either gave rise to the opposite effects (i.e. decrease in the ana- and telophase aberration rates and increase in the modal karyotype frequency) or the aberration rate remained unchanged.

280. ENVIRONMENTAL SAMPLING FOR HAZARDOUS WASTES

Anonymus

No affiliation given

ACS Symp Ser (267):1-132 (1984)

Some of the important considerations in designing and implementing environmental sampling programs for waste disposal sites, with particular attention to surface and subsurface sampling for hazardous wastes, are discussed under the following headings: hazardous waste: questions and issues from the field; uses of environmental testing in human health risk assessment; assessing cyanide contamination from an aluminum smelter; 2,3,7,8-tetrachlorodibenzo-p-dioxin sampling methods; field measurement of polychlorinated biphenyls in soil and sediment using a portable gas chromatograph; using geonics in assessing lead contamination near smelters; lead levels in blood of children around smelter sites in Dallas; an approach to the interdisciplinary design of multifactor experiments; statistical methods in environmental sampling; soil sampling quality assurance and the importance of an exploratory study; quality assurance for a measurement program; new ways of assessing spatial distributions of pollutants; and detecting elevated contamination by comparisons with background.

281. INFLUENCE OF MYCOPLASMA ARGININI INFECTION ON THE INDUCTION OF ARYL HYDROCARBON HYDROXYLASE BY TCDD IN RAT HEPATOMA CELL CULTURES

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Food Chem Toxicol 20(5):599-602 (1982)

Mycoplasma arginini was eliminated from a rat hepatoma cell line (H-4-II-E) by plating at low cell density and treatment with chlorotetracycline (250 ug/ml), kanamycin (250 ug/ml), tylosin (100 ug/ml), 3% M arginini antiserum and 5% fresh guinea-pig serum. The induction of AHH activity in the cell culture was measured in response to increasing concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The ED50 values (estimated doses that produce 50% max enzyme induction) were calculated to be 0.256, 0.452 and 0.344 pmol TCDD/plane for original, mycoplasma-free and reinfected cells, respectively. Although the absence of M arginini in the rat hepatoma cell line makes the cells slightly less responsive to AHH induction by TCDD, this decrease does not detract from the use of the method to screen food extracts and environmental samples for the presence of certain toxic planar organic compounds. (Author abstract) (18 Refs)

282. RADIOIMMUNOASSAY OF CHLORINATED DIBENZO-P-DIOXINS

Allee PW, Luster MI, Chou K, Clark G, McKinney JD
Lab. Environmental Chemistry, Natl. Inst. Environmental Health
Sciences, Res. Triangle Park, NC, 27709
Methods Enzymol. 86:619-628 1982

Preparation of antigens for radioimmunoassay (RIA) of dibenzo-p-dioxins (DPD), production and screening of antisera, principle of RIAs for DPD, tissue extraction and cleanup, preparation of radiolabeled compounds, and the assay procedure are described. Both antibody affinity and avidity vary from animal to animal as well as between primary and secondary response. Assays permitting detection of as little as 30 pg of 2,3,7,8-tetrachlorodibenzo-p-dioxin are obtainable. Other dioxin isomers and some chlorinated dibenzofurans cross-react, but classes of compounds other than these should not interfere. (16 Refs)

283. ASSESSMENT OF MYELOTOKICITY CAUSED BY ENVIRONMENTAL CHEMICALS

Burman GA, Luster MI, Duen JH, Campbell ML
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Research Triangle Park, NC, 27709
Environ Health Perspect. 45:129-135 1982

Potential antineoplastic agents must be screened for the delayed toxicity that occurs in many cases of drug-induced bone marrow aplasia. In vitro clonal assays for hematopoietic progenitor cells have been developed to assess the degree of myelotoxicity. This adverse side effect is often the limiting factor in the development of new cancer chemotherapeutics. In addition, many environmental chemicals are cytotoxic to rapidly proliferating cells, but a systematic assessment of their myelotoxicity has not been performed. We have used clonal marrow assays to investigate a panel of chemicals including 2,3,7,8-tetrachlorodibenzo-p-dioxin, polybrominated biphenyls, diethylstilbestrol, benzo(a)pyrene and indomethacin. All were immunotoxic, some to pleuripotent hemopoietic stem cells and others to granulocyte-macrophage progenitors, and at concentrations below those causing other toxic manifestations. This shows that these bone marrow clonal assays, and hopefully future ones for erythroid, B- and T-lymphocytes, and megakaryocytes, will provide the specificity and sensitivity necessary to delineate the myelotoxicity of a broad spectrum of environmental chemicals. (Author abstract) (35 Refs)

284. CELL-MEDIATED IMMUNITY AND ITS APPLICATION IN TOXICOLOGY

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Environ Health Perspect. 45:31-36 1982

A variety of in vivo and more recently in vitro assays have been described to assess cell mediated immunity (CMI). Two methods routinely employed in our laboratory to assess CMI following exposure to chemicals in rodents included delayed hypersensitivity and in vitro lymphoproliferation. Preliminary studies indicate that depressed delayed hypersensitivity responses, as performed by a radiometric assay, correlates with altered susceptibility to infectious agents and tumor cell challenge following exposure to immunotoxic chemicals. Furthermore, suppression of T-cell lymphoproliferative responses to at least 50% below control values correlated with depressed delayed hypersensitivity responses and altered host susceptibility. On the other hand, when suppression of T-cell lymphoproliferative responses are within 50% of control values, delayed hypersensitivity and host susceptibility parameters are not affected. Assuming adequate technical expertise

and accurate data interpretation, CMI assays of these types can provide a valuable data base for toxicology studies and immunotoxicity assessment etc. (Author abstract) (21 Refs)

285. POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS: A BIOANALYTICAL APPROACH

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Chemosphere. 10:11:19-25 1981

The sensitivity and utility of the receptor binding assay in the bioanalysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and related polychlorinated dibenzo-p-dioxins (PCDDs) was investigated in municipal incinerator fly ash extracts. Extracts were derived from 5 cubic meter samples of gaseous material. The PCDD/polychlorinated dibenzofurans (PCDF) fraction from municipal incinerator fly ash extract avidly binds to the hepatic cytosolic receptor protein which has a high affinity for 2,3,7,8-TCDD and related compounds. The concentration of 2,3,7,8-TCDD in the total PCDD/PCDF fraction from the fly ash was 11.7 nanog. The expected value for the displacement of 3H-TCDD by unlabeled 2,3,7,8-TCDD (EC50 value) was 3.21 nanog per incubation. The EC50 obtained for the fly ash extract which was based only on the concentration of 2,3,7,8-TCDD was 72 picog per incubation. Thus, the extract was approx 45 x more active than expected if the 2,3,7,8-TCDD was the only competing ligand. The increased activity of the fly ash fraction is attributed to the presence of other PCDD and PCDF isomers and congeners which also compete for the hepatic cytosolic protein. (46 Refs)

286. THE USE OF DIOXIN ISOMER GROUP RATIOS TO IDENTIFY SOURCES AND DEFINE BACKGROUND LEVELS OF DIOXINS IN THE ENVIRONMENT

Townsend DL
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J. Environ Sci Health (B). 15(5):571-609 1980

Environmental samples containing a mixture of dioxin isomers showed a characteristic composition indicative of their source and the destruction reactions taking place in the environment. A numerical correlation was found between the various isomer groups. Environmental samples correlated with regard to the relative amounts of octachlorodibenzo-p-dioxin (OCDD) and tetrachlorodibenzo-p-dioxin (TCDD). It was suggested ratios of dioxins continue to hold below-measurable levels of TCDD and that one can obtain a reasonable estimate of the level of TCDD from the levels of OCDD, H7-chlorodibenzo-p-dioxin, and hexachloro-p-dioxin. discovery of any dioxin group implies the existence of others. Lack of specific isomer groups reflects a difference in age, source, or destruction mechanism of sample. It was suggested that once a particle leaves the source and enters a more oxidative environment, the ratio should shift toward the mean value found in nature. Although the absolute concentration of the dioxins found at any one sample point will vary because of the number of particles landing at the location, the relative amount of each dioxin should be independent of the absolute concentration and reflect only the thermal history of the particle. Lower chlorinated dioxins were favored in samples from combustion units. The reduction-oxidation model was applied to observed dioxin ratios following incineration. A kinetic model for degradation of dioxin isomer groups was presented. (1 Ref)

267. REVIEW OF THE DIOXIN PROBLEM. MASS SPECTROMETRIC ANALYSES OF TETRACHLORODIOXINS IN ENVIRONMENTAL SAMPLES

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Dept. Health and Human Services, Food and Drug Admin., Natl. Center for Toxicological Res., Jefferson, AR, 72079

Humal Mass Spectrom. 7:111/121:486-492 1980

Polychlorinated dibenzo-p-dioxin is reviewed, the findings of a Consensus Forum held at the National Center for Toxicological Research are summarized, and the application of atmospheric pressure ionization mass spectrometry for the analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is discussed. The review of polychlorinated dibenzo-p-dioxin includes information on stability and persistence of these compounds in the environment; uptake, accumulation, and translocation in the environment; absorption, tissue distribution, and excretion; toxicity, carcinogenicity, mutagenicity, and teratogenicity, and toxicity to humans. Low resolution mass spectrometry, atmospheric pressure ionization mass spectrometry, and high resolution mass spectrometry used for the detection of TCDD have sensitivities ranging from 1 to 50 ppm. It was the general opinion of the Consensus Forum that each of the techniques is capable of detecting and quantifying TCDD. Because a few ppm of TCDD in the diet can cause fetotoxic and carcinogenic effects, attempts should be made to detect lower levels of TCDD in order to ensure full protection of the public health. (82 Refs)

268. IDENTIFICATION AND QUANTIFICATION OF POLYCHLORINATED DIBENZO-P-DIOXINS (PCDDs) AND DIBENZOFURANS (PCDFs) IN 2,4,5-T-ESTER FORMULATIONS AND HERBICIDE ORANGE

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Dept. Organic Chemistry, Univ. Umea, S-900 87 Umea, Sweden
Chem. pharm. 7:51:431-438 1978

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in samples of esters of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) of European origin and Herbicide Orange (a 1:1 mixture of butyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T) were identified and quantified. An analytical technique using a neutral clean-up involving chromatography on silica and alumina followed by gas chromatography-mass spectrometry using a high-resolution glass capillary column was used. PCDDs were detected in 4/5 2,4,5-T ester samples, 2,3,7,8-tetrachlorodibenzo-p-dioxin being the most abundant in all samples (range 0.1-0.96 microg/g). Two of the samples containing 2,3,7,8-tetra-CDD showed the presence of a 2,3,7,8-tetra-CDF isomer of unknown substitution. All three samples of Herbicide Orange showed the presence of PCDDs at varying levels, the 2,3,7,8-isomer being the major tetra-CDD found in all samples, which was present at 0.12, 1.1 and 5.1 microg/g. One tri-, four tetra- and one penta-CDF isomer were found in one of the Herbicide Orange samples, no PCDFs were found in the other two. (17 Refs)

C.

BIOLOGICAL CONTROL OF ENVIRONMENTAL CONTAMINATION BY DIOXINS AND DIBENZOFURANS

269. THE IDENTIFICATION AND CLONING OF GENES ENCODING HALOAROMATIC CATABOLIC ENZYMES AND THE CONSTRUCTION OF HYBRID PATHWAYS FOR SUBSTRATE MINERALIZATION

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Department de Biochimie Medicale, Centre Medicals Universitaires, Geneva, Switzerland

Bact. Life Sci. 28:47-60 1980

This paper reviews the genetic basis of haloaromatic biodegradation by bacteria, with a focus on the genetic analysis of *Alcaligenes eutrophus* JMP134, an organism which can utilize 3-chlorobenzoate, 2,4-dichlorophenoxyacetate (2,4-D) and related compounds as sole carbon and energy sources, and *Paradomonas* sp. B13, a chlorobenzoate degrader. The involvement of transmissible plasmids pJP4 and pWR1, isolated from strains JMP134 and B13, respectively, in chloroaromatic mineralization has been examined, and restriction fragments of both plasmids have been cloned on the broad host range plasmid vector pKT231. Transposon Tn5 mutagenesis of these and other soil isolates enriched in and purified from mixed cultures utilizing 2,4,5-trichlorophenoxyacetate (2,4,5-T) as sole carbon and energy source, has been carried out using a 'suicide' transposon donor, pLG221 (Colibd6d-1::Tn5). Mapping of Tn5 insertions in mutants which accumulate pathway intermediates has facilitated the identification and cloning of genes encoding chlorocatechol 1,2-dioxygenase, and other key enzymes in haloaromatic catabolism. There are good prospects for the genetic construction of hybrid haloaromatic catabolic pathways by combining genes encoding broad specificity enzymes, capable of transforming halogenated analogues of their natural substrates, with genes for halocatechol degradation. (104 Refs)

270. TRANSPOSON MUTAGENESIS AND CLONING ANALYSIS OF THE PATHWAYS FOR DEGRADATION OF 2,4-DICHLOROPHENOXYACETIC ACID AND 3-CHLOROBENZOATE IN *ALCALIGENES EUTROPHUS* JMP134(pJP4)

Don RH, Weghman AJ, Knackmuss HJ, Tomini KN

Department of Medical Biochemistry, University of Geneva, Switzerland

J. Bacteriol. 161(1):85-90 1983

Plasmid pJP4 permits its host bacterium, strain JMP134, to degrade and utilize as sole sources of carbon and energy 3-chlorobenzoate and 2,4-dichlorophenoxyacetic acid (R. H. Don and J. M. Pemberton, J. Bacteriol. 145:681-686, 1981). Mutagenesis of pJP4 by transposons Tn5 and Tn1771 enabled localization of five genes for enzymes involved in these catabolic pathways. Four of the genes, tddB, tddC, tddD, and tddE, encoded 2,4-dichlorophenol hydroxylase, dichlorocatechol 1,2-dioxygenase, chloromuconase cyclisomerase, and chlorodienelactone hydrolase, respectively. No function has been assigned to the fifth gene, tddF, although it may encode a trans-chlorodienelactone isomerase. Inactivation of genes tddC, tddD, and tddE, which encode the transformation of dichlorocatechol to chloromaleylacetic acid, prevented host strain JMP134 from degrading both 3-chlorobenzoate and 2,4-dichlorophenoxyacetic acid, which indicates that the pathways for these two substrates utilize common enzymes for the dissimilation of chlorocatechols. Studies with cloned catabolic genes from pJP4 indicated that whereas all essential steps in the degradation of 2,4-dichlorophenoxyacetic acid are plasmid encoded, the conversion of 3-chlorobenzoate to chlorocatechol is specified by chromosomal genes.

291. GENETIC AND PHYSICAL MAP OF THE 2,4-DICHLOROPHENOXYACETIC ACID-DEGRADATIVE PLASMID pJP4

Don RH, Pendleton JM

Department of Biochemistry, Centre Medical Universitaire,
Université de Genève, Switzerland
J Bacteriol. 161(1):466-8 1983

Plasmid pJP4 is an 80-kilobase, IncP1, broad-host-range conjugative plasmid of *Alcaligenes eutrophus* encoding resistance to mercuric chloride and phenyl mercury acetate and degradation of 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and 3-chlorobenzoate. By the use of cloning, transposon mutagenesis, and restriction endonuclease analysis, a biophysical and genetic map of pJP4 was generated.

292. OXIDATION OF PERSISTENT ENVIRONMENTAL POLLUTANTS BY A WHITE ROT FUNGUS

Bumpus JA, Tien M, Wright D, Aust SD

Department of Biochemistry, Michigan State University, East
Lansing 48824
Science 220(4706):1454-6 1983

The white rot fungus *Phanerochaete chrysosporium* degraded DDT [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane], 3,4,5'-trichlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl, 2,3,7,8-tetrachlorodibenzo-p-dioxin, lindane (1,2,3,4,5,6-hexachlorocyclohexane), and benzo(a)pyrene to carbon dioxide. Model studies, based on the use of DDT, suggest that the ability of *Phanerochaete chrysosporium* to metabolize these compounds is dependent on the extracellular lignin-degrading enzyme system of this fungus.

293. EVOLUTION AND SPREAD OF PESTICIDE DEGRADING ABILITY AMONG SOIL MICRO-ORGANISMS

Novotny JM, Corns B, Dun PH

Dept. Microbiology, Univ. Queensland, St. Lucia, 4067, Australia
Plasmids of Medical, Environmental and Commercial Importance
Proceedings of the Symposium on Medical, Environmental and
Commercial Importance, held in Spitzing, West Germany, 26-28
April 1979. Toronto: EN. Publ. A. ed. New York: Elsevier/
North-Holland Biomedical Press. Development in Genetics, Vol.
1, 434 pp., 1979.

Evidence is presented which indicates that the evolution of the ability to degrade the chlorinated p-henoxyherbicide 2,4-dichlorophenoxyacetic acid occurs by a process of plasmid borne gene duplication and mutation. It is reported that a major difference between non-persistent and recalcitrant pesticide molecules appears to be the ability to elicit the rapid evolution and spread of pesticide plasmids. (22 Refs)

D.

PUBLIC HEALTH ISSUES AND REGULATIONS

294. HEALTH IMPLICATIONS OF 2,3,7,8-TETRACHLORODIBENZO(DIOXIN) (TCDD) CONTAMINATION OF RESIDUAL SOIL

Kimbrough RD, Falk H, Suter P, Fries G

Center for Environmental Health Centers for Disease Control,
Atlanta, Georgia
J Toxicol Environ Health 14(1):47-55 1984

Extrapolations from animal toxicity experiments (including carcinogenicity and reproductive effects) to possible human health effects can be used to estimate a reasonable level of risk for 2,3,7,8-tetrachlorodibenzo(dioxin) (2,3,7,8-TCDD). Extrapolations are derived from: (1) review of published studies, (2) a complex set of assumptions related to human exposure to contaminated soil, and (3) estimates of (a) a dose response curve, (b) appropriate margins of safety, and/or (c) applicable mechanisms of action. One ppb of 2,3,7,8-TCDD

in soil is a reasonable level at which to begin consideration of action to limit human exposure for contaminated soil. (126 Refs)

295. LESSONS FOR LIVING IN A CHEMICAL WORLD (I) (Eds)

Selhub U

Environmental Sciences Laboratory, Mount Sinai School of Medicine,
New York
Bull Environ Contam Toxicol 33(6):582-95 1984

Keywords (MeSH): Asbestos/etiology; Breast Feeding/drug effects; Child; Construction Materials/adverse effects; Dioxins/*adverse effects; Disease/*chemically induced; Environmental Pollutants/*adverse effects; Female; Human; Lung Neoplasms/chemically induced; Male; Mesothelioma/chemically induced; Neoplasms/chemically induced; Occupational Diseases/chemically induced; Review; Risk; Smoking; United States Environmental Protection Agency; United States

296. THE PUBLIC HEALTH RESPONSE TO 2,3,7,8-TCDD ENVIRONMENTAL CONTAMINATION IN MISSOURI

Sutro PA, Farney D, Stern G, Donnell HD, Falk H, Huchins R,
Spratlen WA, Sampson E, Smith SJ

Center for Environmental Health, Centers for Disease Control,
Atlanta, GA 30333
Public Health Rep 100(3):289-95 1985

In 1971, waste oil containing 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was sprayed for dust control on a number of residential, recreational, and work areas in Missouri. In several of them, the level and extent of environmental contamination were not known until late 1982 or 1983. Extrapolation from existing toxicological data indicated the potential for substantial adverse health effects in highly exposed populations. As a result, the Missouri Division of Health and the Centers for Disease Control initiated close collaboration with the Environmental Protection Agency (EPA) on review and evaluation of environmental data, the development of health advisories to EPA on the need for remedial or preventive actions at specific contaminated sites, a health education effort for the medical community and general public, establishment of a dermatological screening clinic, establishment of a central listing of potentially exposed persons through administration of a health effects survey questionnaire, and a pilot medical study of a 'highest risk' cohort. Strategies for additional interventions will continue to be based on findings derived from this first phase of the investigation.

297. THE GENOTOXIC/EPIDEMIOLOGIC DISTINCTION: RELEVANCE TO CANCER POLICY

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Division of Environmental Health Sciences, Columbia University
School of Public Health, New York, New York
Environ Res 34(1):175-91 1984

Should federal agencies use separate, less stringent guidelines for regulating epigenetic or nongenotoxic carcinogens on the assumption that thresholds are likely to exist for these agents? This article reviews recent initiatives by the Environmental Protection Agency that either propose or informally adopt this approach in light of responses from the scientific community and a review of the recent literature. Relevant background is provided by current research concerning the role of chromosomal damage and oncogene activation in carcinogenesis along with findings that classical promoters or 'epigenetic' agents can induce both DNA damage and chromosomal rearrangements. The conclusion is that such a revision of cancer policy is not now supported by available scientific data concerning chemical carcinogenesis. (88 Refs)

298. THE CURRENT USE OF STUDIES ON PROMOTERS AND COCARCINOGENS IN QUANTITATIVE RISK ASSESSMENT

Saura JF, Maderly D, McGaughey R, Durkin P, Dearson ML
Environmental Criteria and Assessment Office, U.S. EPA,
Cincinnati, OH
Environ Health Perspect. 30:339-68 (1983)

Several of the priority pollutants discussed in EPA's Ambient Water Quality Criteria documents have been reported to have promotion or cocarcinogenic activity. For example, phenol appears to have tumor-promoting activity in mice when repeatedly applied after initiation with either 7,12-dimethyl-1,2-benzanthracene (DMBA) or benzo(a)pyrene (BaP). Similarly, it has been reported that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent promoter of liver tumors as well as a cocarcinogen. However, in developing guidelines to derive ambient water quality criteria, it became apparent that satisfactory approaches had not been developed for using promotion/cocarcinogen data in human health risk estimation, nor were available promotion and/or cocarcinogen data on individual chemicals strong enough to permit a defensible quantitative risk estimation, if such approaches had existed. For this reason, the criteria derived for pollutants with reported promotion/cocarcinogenic activities were based on approaches for carcinogenic (e.g., TCDD), toxic (e.g., fluoranthene) or organoleptic effects (e.g., 2,4-dichlorophenol). Nonetheless, with advances in studies on both the biological mechanisms and dose/response patterns of promoters and cocarcinogens, it may be possible to develop a scientifically valid quantitative approach to use this type of data for derivation of ambient water quality criteria or other risk assessments. Some progress toward this goal and the problems associated with this effort are discussed.

299. CHEMICALS, CANCER AND BIRTH DEFECTS

Bakken A
ICI Australia Operations Pty. Ltd., Ascot Vale, Victoria, Australia
Search. 12(1/2):96-7 (1981)

Public debate over the risks associated with the use of chemicals has been largely one-sided: antagonists to chemicals have received wide and favorable publicity, while their advocates have had little media support. Far too much attention has been given to the adverse effects of synthetic products than to those of traditional foods and practices. It is shown that on a strictly numerical basis, the hazards of eating peanut butter (containing aflatoxins) is much greater than those of drinking saccharin-sweetened soft drinks; peanuts grow naturally, while saccharin is a man-made chemical. The contradictions in current regulations of pesticides and food additives are discussed. Chemicals and birth defects are discussed with respect to exposure to dioxin in the pesticide 3,4,5 T. This herbicide can be used safely, but will probably be banned for political reasons in most countries. The public reaction in the United States against the proposed banning of saccharin illustrates that the public can evaluate the risks vs benefits and can have a voice in the regulation of chemicals in the environment. (31 Refs)

300. STORAGE AND DISPOSAL OF WASTE MATERIAL; PROHIBITION OF DISPOSAL OF TETRACHLORODIBENZO-P-DIOXIN

Environmental Protection Agency
401 M St. SW, Environmental Protection Agency, Washington, DC, 20460
Fed Regist. 47(198):12676-12687 (1982)

The final ruling on storage and disposal of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) a fetotoxic, teratogenic, and carcinogenic compound produced by Vertac Chemical Company is presented. Vertac is prohibited from disposing of wastes

containing TCDD produced at Jacksonville, Arkansas before the date of the ruling. Other persons must notify the Environmental Protection Agency 60 days in advance of any planned disposal of TCDD. (25 Refs)

301. DIOXINS, VOLUME I. SOURCES, EXPOSURE, TRANSPORT, AND CONTROL

Exposito MP, Drake HM, Smith JA, Owen TV
PEDCo-Environmental, Inc., Cincinnati, OH
Dioxins: Volume I. Sources, Exposure, Transport, and Control.
Available from National Technical Information Service,
Springfield, VA, as PB80-220072, EPA-600/2-80-156, 270 pp., 1980

Concern about the potential contamination of the environment by dibenzo-p-dioxins through the use of certain chemicals and disposal of associated wastes prompted this study. The volume reviews the extensive body of dioxin literature that has recently become available. (Author abstract)

VII. REVIEWS

A. CARCINOGENICITY

302. CARCINOGENICITY OF ORGANIC HALOGENATED COMPOUNDS

Gross H, Wolff T
Gesellschaft für Strahlen- und Umweltforschung München, Institut
Toxikologie und Biochemie, D-8042 Neuherberg, Germany
ACS Monogr. 182:525-75 (1984)

Halogenated hydrocarbons are encountered in numerous occupational and nonoccupational situations; therefore, their carcinogenic potential is a critical concern. Such hydrocarbons are starting materials, intermediates, and by-products for organic syntheses. They are used as fire retardants, solvents, and pesticides and can be found in many commercial products. Although the exact mechanism of chemical carcinogenesis is not clear, two major categories of carcinogens can be distinguished: those acting by genotoxic mechanisms and those acting by epigenetic mechanisms. The differentiation between genotoxic and epigenetic carcinogens requires information on their metabolism; tissue distribution; route and rate of excretion; mutagenicity, especially in the various in vitro systems; interaction with cellular macromolecules, such as proteins or nucleic acids; organ-specificity of cytotoxic effect; and promoting activity. The toxicities and carcinogenicities of specific halogenated compounds are reviewed under the following general headings: agents for organic synthesis, fire retardants, miscellaneous agents, polychlorinated biphenyls, and pesticides. The compounds discussed comprise a large and heterogeneous group of chemicals that differ greatly in structure, metabolism, and biological activity, it is, therefore, fitting that they also differ in their modes of carcinogenic action. Some of the compounds clearly are initiators of tumor formation, i.e., genotoxic agents, whereas others apparently operate by epigenetic mechanisms, such as recurrent organ-specific cytotoxicity, for some compounds both mechanisms might be operative, while for others data are lacking to make a distinction. The problems of interpreting carcinogenicity data are obviously not specific for the organic halogenated compounds; however, they are particularly pressing for this group of substances. Many of the compounds do not exert their effects by overtly attacking DNA but by some other, poorly specified mechanism. Furthermore, this group

contains a large number of chemicals that are widely distributed in the environment and are of great economical value. A particular problem arises from the evaluation of positive results that can only be obtained at very high doses of the test compounds. Although there is a need and a scientific basis for using the maximum tolerable dose in bioassays for carcinogenicity, it has to be realized that a high concentration of these chemicals may exert effects profoundly different from those at low concentrations; still, clarification of the mechanism of chemical carcinogenesis remains the major issue. (459 Refs)

303. CELLULAR MECHANISMS FOR TUMOR PROMOTION AND ENHANCEMENT (MEETING ABSTRACT)

Slaga TJ

The Univ. of Texas System Cancer Center, San Antonio, TX 78291

Symposium on Tumor Promotion and Enhancement in the Etiology of Human and Experimental Respiratory Tract Carcinomas, June 18-20, 1986, Williamsburg, VA, U.S. Environmental Protection Agency, p. 1986

Tumor promotion and enhancement appear to play critical roles in carcinogenesis in a number of tissues, such as the skin, liver, bladder, respiratory system, colon, esophagus, stomach, mammary gland, pancreas, and cells in culture. In these systems, a wide variety of promoting or enhancing agents have been found such as diet, diterpenes, fatty acids, peroxides, indole alkaloids, polyacetates, surface-active agents, long-chain alkanes, tobacco smoke condensate, anthralin, chrysarobin phenols, dioxins, phenobarbital, hormones, bile acids, saccharin, cyclamate, tryptophan, butylated hydroxytoluene, polychlorobiphenyls, polybromobiphenyls, oral contraceptives, and DDT. Presently, no common mechanism has been found for these diverse promoting and enhancing agents except that they bring about the expansion of the initiated cells in a target tissue through their effects on cell proliferation and differentiation. Much of our knowledge about the cellular and molecular mechanisms of tumor promotion has come from studies using the phorbol ester tumor promoters in the mouse skin two-stage model and in various cell culture systems. Some skin tumor promoters, such as the phorbol esters, indole alkaloids, and polyacetates appear to act through a membrane receptor (protein kinase), whereas other types, such as peroxides, may act through a free radical mechanism. In both cases, important membrane changes occur. The major effect of the skin tumor promoters, regardless of the type, is the specific expansion of the initiated cells in the epidermis. This appears to occur by both direct and indirect mechanisms. The skin tumor promoters appear to have a direct effect on the initiated cells, possibly by mimicking some endogenous growth factor, which leads to an increased growth potential and/or a decreased differentiation capacity of the initiated cells. An inhibition of cell-cell communication and stimulation of differentiation of non-initiated cells appear to be important indirect mechanisms of further expanding the initiated cell population. A number of other cellular and molecular effects of tumor promoters, such as stimulation of prostaglandin and polyamine synthesis, gene amplification, and chromosomal changes, also appear to play a role in tumor promotion. The studies, which reveal that skin tumor promotion can be operationally and mechanistically further divided into several stages, suggest that many of the cellular and molecular events occur in a specific sequential manner in which certain inhibitors are very specific for certain stages of promotion.

304. REPORT OF ICR/MC TASK GROUP 5 ON THE DIFFERENTIATION BETWEEN GENOTOXIC AND NON-GENOTOXIC CARCINOGENS (312 Refs)

Anonymous

Mutat Res. 133(1):1-49 1984

Keywords (MeSH): Animal; Butylated Hydroxytoluene/toxicity; Carcinogens/*toxicity; Cell Line; Dieldrin/toxicity; Dioxins/toxicity; Drug Screening/methods; DDT/toxicity; Liver Neoplasms, Experimental/chemically induced; Human; Liver Neoplasms/chemically induced; Mutagenicity Tests/methods; Mutagens/*toxicity; Neoplasms/etiology; Phenobarbital/toxicity; Review; Structure-Activity Relationship.

305. DIOXINS AND DIBENZOFURANS IN CARCINOGENESIS (ONCOLOGY OVERVIEW)

International Cancer Research Data Bank

NCI, Building 82, Room 105, Bethesda, MD 20892

Dioxins and Dibenzofurans in Carcinogenesis (Oncology Overview)

Slaga, T.J. of This Title is Available Through National Technical Information Service, Springfield, VA, as NTIS/DB-P002-922905, 36 pp., 1982.

ONCOLOGY OVERVIEWs are a service of the International Cancer Research Data Bank (ICRDB) Program of the National Cancer Institute. They are retrospective bibliographies containing 100-500 selected abstracts of published research on topics of high interest to cancer investigators. This OVERVIEW includes all aspects of the toxicity, carcinogenicity, cocarcinogenicity, tumor promoting activity and anticarcinogenicity of dioxins and dibenzofurans as well as the effects of these agents on enzyme induction, especially those enzymes involved in the metabolism of chemical carcinogens. The role of the binding of dioxins and dibenzofurans to specific cytosol receptors in the induction of aryl hydrocarbon hydroxylase is also included. In addition, the environmental occurrence of dioxins and dibenzofurans and the effect of human exposure are presented.

306. MUTAGENIC AND CARCINOGENIC EFFECTS OF PESTICIDES

Saleh MA

Dept. Agricultural Biochemistry, Faculty Agriculture, Univ. Cairo, Giza, Egypt

J Environ Sci Health (B). 15(6):507-527 1980

Mutagenic and carcinogenic effects, mode of action, and structure of various classes of active organic pesticides, including halogenated hydrocarbons, miticides, nematocides, and fumigants, are reviewed. Short-term assays for carcinogenic activity include unscheduled DNA synthesis, sister chromatid exchange, and mammalian cell mutagenesis and transformation; the Ames Salmonella test is the most widely used. In general, the halogenated hydrocarbons are usually hazardous, with the insecticidal fumigants (eg, chloroform, carbon tetrachloride, ethylene dibromide, and dibromochloropropane) causing sterility and the chlorinated hydrocarbon insecticides (eg, aldrin, dieldrin, lindane, mirex, chlordane, strobane, and kepone) showing marginal or definite carcinogenic activity. Several organophosphorus insecticides have been shown to be mutagens (eg, trichlorophen, phthalophen, methyl parathion, phosphamidon, desmethyl-trichlorophen, dimethoate, azinphosmethyl, menazon, superacid, monocrotophos, and chlorpyrifos) and some have been shown to be carcinogens. Herbicide impurities tetrachlorodibenzodioxin and maleic hydrazide have demonstrated carcinogenic activity in experiments with mice and rats. Studies have suggested that DNA is the critical target for pesticide carcinogenic activity; the reactive forms of carcinogens are generally electrophiles, which attack nucleophilic sites within the DNA. (90 Refs)

307. NATIONAL CANCER PROGRAM SPECIAL COMMUNICATION

Higman J. DeVita VT
International Agency for Res. on Cancer, World Health
Organization Publications Center, 49 Sheridan Ave., Albany,
NY, 12212
Clin Toxicol. 17(3):477-489 1980

Monographs prepared by the International Agency for Research on Cancer of the World Health Organization that deal with the 'Evaluation of the Carcinogenic Risk of Chemicals to Humans' are described. The first 20 volumes contain evaluations or re-evaluations of 442 chemicals. Monographs review N-nitroso compounds, inorganic and organometallic compounds, polycyclic aromatic hydrocarbons, aromatic amines, organochlorine pesticides, sex hormones, nitrofurans, aromatic azo dyes, aziridines, mustards, natural products, epoxides, carbamates, thiocarbamides, carbazides, pharmaceutical drugs, asbestos, herbicides and dioxins, polychlorinated and polybrominated biphenyls, monomers and plastics, halogenated hydrocarbon pesticides, and solvents. A supplement has been issued to volumes 1-20 that reviews available human data for 54 chemicals, groups of chemicals, or industrial processes identified in the monographs as having human carcinogenicity data. Chemicals are also listed for which animal studies have provided evidence for carcinogenicity, but for which data concerning human exposure is not available. (22 Refs)

B.

MECHANISMS

308. THE REGULATION OF CYTOCHROME P-450 GENE EXPRESSION

Whitlock JP
Ann Rev Pharmacol Toxicol. 20:555-579 1980

309. STUDIES ON THE MECHANISM OF ACTION OF HALOGENATED AROMATIC HYDROCARBONS

Poland A, Knutson J, Gloor E
McArdle Laboratory for Cancer Research, University of Wisconsin,
Madison
Clin Physiol Biochem. 5(2-3):147-54 1980

The halogenated aromatic hydrocarbons (dibenzo-p-dioxins, dibenzofurans, azo(xy)benzenes and biphenyls), a group of toxic chemicals in the environment, (a) are approximate isomers, (b) produce a similar pattern of biologic responses and (c) appear to act by a common mechanism. These compounds reversibly bind to a soluble receptor protein to initiate a coordinate gene expression, analogous to the action of steroid hormones. This receptor controls two distinct and dissociable pleiotropic responses: (a) the induction of microsomal monooxygenase activity and other drug-metabolizing enzymes and (b) morphologic (i.e. toxic) changes, many of which involve altered cell proliferation and/or differentiation in epithelial tissues. (14 Refs)

310. IMMUNOTOXICOLOGY (36 Refs)

Miller K
British Industrial Biological Research Association, Carshalton,
Surrey, U.K.
Clin Exp Immunol. 43(2):219-25 1980

Keywords (MeSH): Animal, Asbestos/adverse effects, Drug Hypersensitivity, Human, Immune Tolerance/drug effects, Immunity, Cellular/drug effects, Immunocompetence/*drug effects, Immunologic Deficiency Syndromes/immunology, Immunosuppressive Agents/adverse effects, Lung Neoplasms/chemically induced, Mice, Neoplasms/chemically induced/immunology, Polybromobiphenyl Compounds/adverse effects, Rats, Review, T Lymphocytes/immunology, Tetrachlorodibenzo(dioxin)/adverse effects

311. PCBs: STRUCTURE-FUNCTION RELATIONSHIPS AND MECHANISM OF ACTION

Sely J, Bandaru J, Sawyer T, Robertson L, Safe L, Parkinson A, Thomas PE, Ryan DE, Roth LM, Lown W, et al
Department of Physiology and Pharmacology, College of Veterinary
Medicine, Texas A and M University, College Station 77843
Environ Health Perspect. 60:47-56 1983

Numerous reports have illustrated the versatility of polychlorinated biphenyls (PCBs) and related halogenated aromatics as inducers of drug-metabolizing enzymes and the activity of individual compounds are remarkably dependent on structure. The most active PCB congeners, 3,4,4',5-tetra-, 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl, are substituted at both para and at two or more meta positions. The four coplanar PCBs resembled 3-methylcholanthrene (3-MC) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) in their mode of induction of the hepatic drug-metabolizing enzymes. These compounds induced rat hepatic microsomal benzo(a)pyrene hydroxylase (aryl hydrocarbon hydroxylase, AHH) and cytochromes P-450a, P-450c and P-450d. 3,4,4',5-Tetrachlorobiphenyl, the least active coplanar PCB, also induced dimethylaminoazobenzene N-demethylase and cytochromes P-450b+e and resembled Aroclor 1254 as an inducer of the mixed-function oxidase system. Like Aroclor 1254, all the mono-ortho- and at least eight di-ortho-chloro analogs of the coplanar PCBs exhibited a 'mixed-type' induction pattern and induced microsomal AHH, dimethylaminoazobenzene N-demethylase and cytochromes P-450a-P-450e. Quantitative structure-activity relationships (QSARs) within this series of PCBs were determined by comparing their AHH induction potencies (EC50) in rat hepatoma H4-II-E cells and their binding affinities (ED50) for the 2,3,7,8-TCDD cytosolic receptor protein. The results showed that there was an excellent correlation between AHH induction potencies and receptor binding avidities of these compounds and the order of activity was coplanar PCBs (3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyls) greater than 3,4,4',5-tetrachlorobiphenyl approximately mono-ortho coplanar PCBs greater than di-ortho coplanar PCBs. It was also apparent that the relative toxicities of this group of PCBs paralleled their biological potencies. The coplanar and mono-ortho coplanar PCBs also exhibit differential effects in the inbred C57BL/6J and DBA/2J mice. These compounds induce AHH and cause thymic atrophy in the former 'responsive' mice whereas at comparable or higher doses none of these effects are observed in the nonresponsive DBD/2J mice. Since the responsiveness of these two mice strains is due to the presence of the Ah receptor protein in the C57BL/6J mice and in relatively low concentration in the DBA/2J mice, the results for the PCB congeners support the proposed receptor-mediated mechanism of action. (79 Refs)

312. POLYCHLORINATED BIPHENYLS (PCBs) AND POLYBROMINATED BIPHENYLS (PBBs): BIOCHEMISTRY, TOXICOLOGY, AND MECHANISM OF ACTION

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University, College Station
CRC Crit Rev Toxicol. 13(4):319-95 1984

Polychlorinated and polybrominated biphenyls are industrial chemical mixtures which have been implicated in numerous human poisonings in Taiwan and Japan (PCBs) and Michigan (PBBs). Moreover, these polyhalogenated biphenyls have been widely detected in the environment including the air, water, fish, wildlife, human adipose tissue, and blood and breast milk. A major problem associated with the analy-

sis and toxicology of this group of chemicals is their chemical complexity (e.g., there are 209 possible PCB isomers and congeners) and the remarkable effects of structure on activity. This article will discuss the effects of structure on the biologic and toxic effects of individual PCB and PBB congeners as well as reconstituted mixtures. The results clearly show that like 'dioxin' (or 2,3,7,8-TCDD), the PCBs and PBBs elicit their effects through a cytosolic receptor protein which preferentially binds with the toxins which are approximate isomers of 2,3,7,8-TCDD. The evidence for this mechanism of action will be discussed in detail. (537 Refs)

513. BIOLOGICAL MECHANISMS OF DIOXIN ACTION

Poland A, Kneibrough RD

Biological Mechanisms of Dioxin Action. Poland A, Kneibrough RD (Eds.). Cold Spring Harbor Laboratory, Brookhaven Report No. 58, 1984

514. THE EFFECTS OF HYPOLIPIDEMIC PEROXISOME PROLIFERATORS ON THE INDUCTION OF SISTER CHROMATID EXCHANGES (29 Refs)

Lorenson H

Institute of Occupational Health, Department of Industrial Hygiene and Toxicology, Helsinki, Finland
Basic Life Sci. 29 Pt 2:965-74 1984

Keywords (MeSH): Animal; Antilipemic Agents/adverse effects/pharmacodynamics; Bone Marrow/drug effects; Carcinogens/pharmacodynamics; Carcinogens, Environmental/adverse effects; Cell Transformation, Neoplastic/chemically induced; Cells, Cultured; Clofibrate/*pharmacodynamics; Crickets; Female; Glycolates/*pharmacodynamics; Hamsters; Herbicides/pharmacodynamics; Human; Lymphocytes/drug effects/ultrastructure; Microbodies/*drug effects; MCPA/*pharmacodynamics; Occupational Diseases/chemically induced; Pest Control; Rats; Review; Sister Chromatid Exchange/*drug effects; 2,4-Dichlorophenoxyacetic Acid/*pharmacodynamics.

515. THE EFFECT OF THE PHENOXYACETIC ACID HERBICIDES 2,4,5-TRICHLOROPHOXYACETIC ACID AND 2,4-DICHLOROPHOXYACETIC ACID AS ASCERTAINED BY DIRECT EXPERIMENTATION

Fagan E, "Silva" J

Residue Rev. 32:29-38 1984

Keywords (MeSH): Animal; Behavior; Animal/drug effects; Chromosomes/drug effects; DNA/biosynthesis; Embryo/drug effects; *Environmental Pollution; Enzymes/metabolism; Female; Fertility/drug effects; Fetus/drug effects; Herbicides/*toxicity; Human; Metabolism/drug effects; Mutagens; Neoplasms/chemically induced; Oogenesis/drug effects; Pregnancy; Teratogens; 2,4-Dichlorophenoxyacetic Acid/*toxicity; 2,4,5-Trichlorophenoxyacetic Acid/*toxicity.

516. 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND RELATED HALOGENATED AROMATIC HYDROCARBONS: EXAMINATION OF THE MECHANISM OF TOXICITY

Poland A, Knutson JC

Ann Rev Pharmacol Toxicol. 22:517-534 1982

In this review, we have examined the biochemical and toxic responses produced by halogenated aromatic hydrocarbons and have tried to develop a model for their mechanism of action. These compounds bind to a cellular receptor and evoke a sustained pleiotropic response. In many tissues this response consists of the expression of a battery of enzymes which are, for the most part, involved in drug metabolism, but in other tissues, those which develop toxicity, an additional set of genes is expressed which effects cellular involution, division, and/or differentiation. The toxicity of these compounds appears to be due to the sustained expression of

a normal cellular regulatory system, of which we were previously unaware. In future investigations it is hoped that we will learn the nature and physiologic role of this regulatory system. Only then can we hope to understand the mechanism of toxicity of these compounds.

517. INDUCTION OF THE DRUG-METABOLIZING ENZYMES (56 Refs)

Poland A

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison

IARC Sci Publ. (52):151-64 1982

Keywords (MeSH): Aldehyde Oxidoreductases/metabolism; Animal; Cytochrome P-450/*metabolism; Enzyme Induction/drug effects; Epoxide Hydrolases/metabolism; Glutathione Transferases/metabolism; Human; Methylcholanthrene/pharmacodynamics; Mice; Microsomes, Liver/*enzymology; Mixed Function Oxidases/*metabolism; Oxidoreductases/*metabolism; Phenobarbital/pharmacodynamics; Quinone Reductases/metabolism; Rats; Review; Tetrachlorodibenzo-dioxin/pharmacodynamics; UDP Glucuronosyltransferase/metabolism.

518. THE BIOLOGICAL RELEVANCE OF IMMUNE SUPPRESSION AS INDUCED BY GENETIC, THERAPEUTIC, AND ENVIRONMENTAL FACTORS. PAPERS PRESENTED AT A WORKSHOP HELD IN WILLIAMSBURG, VA, 13-14 NOVEMBER, 1979

Davis JM, Paderisburgh M

Immunology Section, Environmental Biology Branch, Natl. Inst. Environmental Health Science, Research Triangle Park, NC
The Biological Relevance of Immune Suppression as Induced by Genetic, Therapeutic, and Environmental Factors: Papers presented at a Workshop held in Williamsburg, VA, 13-14 November, 1979. New York: Van Nostrand Reinhold Company, 516 pp., 1981.

Practical applications of immunology to toxicology, environmental health, and regulatory programs are discussed. Topics are discussed under the following headings: the biology of immune suppression and regulation (intrinsic and extrinsic regulation of immune responses immunopharmacology of immunotherapy, heterogeneous interaction of cytotoxic immunodepressants with immunocyte subpopulations), measurements of immunologic parameters (measurement and assessment of immune competency, effects of anti-cancer therapy on various host defense functions in cancer patients, assessment of immune parameters of transplant recipients on immunosuppressive therapy, lymphoproliferative responses to alloantigens by postoperative patients with Stage I lung and breast cancer and their application to prognosis, investigation of the immunobiological effects of polybrominated biphenyls in Michigan farmers, and the potential of high resolution protein mapping as a method of monitoring the human immune system), experimental changes in immunologic parameters following exposure to environmental agents (immunological alterations in mice following acute adult exposure to diethylstilbestrol, effects of alkylating agents on the immune response, modulation of the immune response in laboratory animals by lead and cadmium, direct and induced suppression of in vitro antibody production by selected phenols, selective action of alkylating agents on helper and suppressor functions), host susceptibility and lung defense mechanisms (host resistance models as endpoints for assessing immune alteration following chemical exposure to diethylstilbestrol, cyclophosphamide, and 2,3,7,8-tetrachlorodibenzo-P-dioxin; influence of antibiotics on host defense; effects of environmental chemicals on host defense responses of the lung; approaches for studying immunity in deep lung and ef-

fects of corticosteroids; and a unifying model for immunotoxicology). (363 Refs)

319. THE GENETIC TOXICOLOGY OF PHENOXY ACIDS OTHER THAN 2,4,5-T

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Swiss Federal Res. Station, CH-8620 Wädenswil, Switzerland

Mutat Res. 55:5/6:197-226 1978

Herbicides of the phenoxy acid type other than 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are reviewed. The uses of these herbicides and their toxicology and metabolism are discussed. The review includes information on their genetic effects, their influences on nucleic acids, studies in microorganisms, studies in plants, investigations on *Drosophila*, and mammalian testing. It is concluded that phenoxy acids interact with nucleic acid metabolism, they are weak mutagens in many different systems, they do not easily penetrate cell membranes, they are suspected as carcinogens, and they do not normally leave large residues on edible products, which means that the average consumer will not be heavily exposed, but people working with these herbicides should still be careful in handling them. It is recommended that their use be restricted to areas where their application can be strictly controlled, the protection of people working with them should be evaluated and possibly improved, their penetrability through membranes of different cell types including germ cells should be investigated, and the carcinogenicity of 4-chlorophenoxyacetic acid and 4-(2,4-dichlorophenoxy)butyric acid should be tested by the best available techniques. Mutagenicity tests in which target concentrations of phenoxy acids can be measured are needed. The possible interactions between phenoxy acids and the solvents and stabilizers used in formulated end-products should be investigated. (133 Refs)

C.

OCCURRENCE AND PHARMACOKINETICS

320. SOURCES AND FATE OF POLYCHLORINATED DIBENZODIOXINS, DIBENZOFURANS AND RELATED COMPOUNDS IN HUMAN ENVIRONMENTS

Tiersten TD, Taylor ML, Garrett JM, VanNess GF, Selik JG

Wagel DJ, Ferguson GL, Schecter A

Environ Health Perspect. 50:145-158 1983

Several of the major incidents resulting in potential human exposures to polychlorinated dibenzodioxins (PCDDs) and/or polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and related compounds which have occurred in the U.S. in recent periods have resulted from improper disposal of hazardous chemical wastes. Prominent examples of such environmental contamination episodes are the Love Canal, into which ton quantities of chlorinated organic compounds containing substantial concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were deposited; numerous sites in the State of Missouri which were contaminated by the dumping of chlorinated organic wastes containing various PCDDs and possibly PCDFs, and PCBs; and the environs of a 2,4-D/2,4,5-T manufacturing plant site in Arkansas, which were contaminated with PCDDs. Environmental assessments of such sites have revealed extensive contamination of soils, waterways, fish and other biological species with these toxic compounds, which in turn could lead to human exposures. Other recently identified sources of PCDDs, PCDFs and related compounds in human environments include stack effluents from municipal refuse incineration, and fires and explosions involving electrical devices containing PCBs and polychlorinated benzenes. Data obtained in assessments of such incidents are presented, and the

implication of these findings with respect to the distribution and persistence of PCDDs, PCDFs and related chemicals in the environment and possible effects on humans are discussed.

321. ANALYSIS OF POLYCHLORINATED DIOXINS AND FURANS

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Environ Sci Technol. 18(3):784-90A 1984

This review considers polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), two series of tricyclic aromatic compounds that exhibit similar physical, chemical and biological properties. Studies have suggested that there is a pronounced difference in toxic biological effects among the different PCDD and PCDF isomers. Toxicities can vary by a factor of 1000 to 10,000 for very closely related isomers. Those with the highest carcinogenic activity and toxicity have 4,6 chlorine atoms and all lateral (2,3,7,8) positions substituted with chlorine. Many analytical methods have been developed for the identification of trace amounts of PCDDs and PCDFs, the most specific of which are based on mass spectrometry. These isomer-specific techniques have enabled all 75 PCDDs and 135 PCDFs to be identified. With respect to the analytical methods, procedures for sample cleanup, isomer identification and quantification are discussed. The formation of PCDDs and PCDFs, both in the lab and in the environment, are described. Some commercial products containing PCDDs and PCDFs are discussed, such as phenoxy herbicides, hexachlorophene, chlorophenols and polychlorinated biphenyls. A section is included on levels in human samples as a result of occupational or accidental exposure, along with a consideration of the carcinogenic effects of dioxins and dibenzofurans. A very limited number of studies suggest that exposure to these compounds increases the risk of soft-tissue sarcoma, Hodgkin's disease, non-Hodgkin's lymphomas and, in cases where the compounds are breathed, nasal cancer. (85 Refs)

322. MECHANISM CONTROLLING THE RENAL EXCRETION OF XENOBIOTICS IN FISH: EFFECTS OF CHEMICAL STRUCTURE (35 Refs)

Prichard JB, Bend JR

Laboratory of Pharmacology, NIH/NIEHS, Research Triangle Park, North Carolina

Drug Metab Res. 15(4):655-71 1984

Keywords (MeSH): Animal; Benzo(a)pyrene/metabolism; DDT/metabolism; Fishes/*metabolism; Kidney/*metabolism/physiology; Review; 2,4-Dichlorophenoxyacetic Acid/metabolism.

323. DOSE-DEPENDENT PHARMACOKINETICS: EMPHASIS ON PHASE I METABOLISM (301 Refs)

Davies PG, Sander JE

Western Psychiatric Institute and Clinic, University of Pittsburgh, Pennsylvania

Drug Metab Res. 14(1):347-405 1983

Keywords (MeSH): Adolescence; Adult; Age Factors; Aged; Animal; Antineoplastic Agents/metabolism; Child; Child, Preschool; Coumarins/metabolism; Dioxanes/metabolism; Dose-Response Relationship; Drug; Drugs/*metabolism; Human; Infant; Infant, Newborn; Kinetics; Mathematics; Middle Age; Phenylbutazone/metabolism; Phenytoin/metabolism; Review; Tissue Distribution; 2,4,5-Trichlorophenoxyacetic Acid/metabolism.

324. THE SOURCES AND ROUTES OF CHILDHOOD CHEMICAL EXPOSURES

Rogan WJ

Biochemistry Branch, Natl. Inst. Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC, 27709
J. Pediatr. 97:51-60/1-60/5 1980

Exposure conditions examined include: toxic chemicals carried home by parents returning from work; exposure of mother and fetus to toxic chemicals at the mother's place of employment; air pollution hazards; concentrations of toxic chemicals in mothers' milk; bioaccumulation of chemicals in lower stages of the food chain which in turn are ingested by humans; accumulation of chemicals in various parts of the human body; pollution in the home particularly arising from renovations or inappropriate use of chemicals; and exposures of epidemic proportions resulting from accidental spillages, massive misuses of chemicals, or improper storage of chemical wastes. Specific references to cancer included the development of *neuroblastoma* in children who had been playing in mine tailings around asbestos sites, the development of angiosarcoma following vinyl chloride exposure, and lung cancer in response to toxic irritants. (49 Refs)

325. RELATIONSHIP BETWEEN BIOTRANSFORMATION AND THE TOXICITY AND FATE OF XENOBIOTIC CHEMICALS IN FISH

Lach JJ, Bend JR

Dept. Pharmacology and Toxicology, The Medical Coll. Wisconsin, Milwaukee, WI, 53226
Environ. Health Perspect. 34:115-131 1980

Some of the more recent developments in the study of xenobiotic (XB) chemicals in aquatic species are reviewed, and the relationship between biotransformation and the toxicity, persistence, and bioaccumulation of XB chemicals in fish is discussed. As to the metabolism of XB in living fish, it appears that oxidation generally proceeds more slowly than either hydrolysis or conjugation. Several studies illustrate that biotransformation may be an important determinant of the toxicity of certain chemicals in fish and that it may play a role in species sensitivity to chemicals. There is limited evidence that biotransformation rates may affect the bioaccumulation of certain compounds. It appears that compounds which are conjugated directly or readily metabolized to materials which can be conjugated with glucuronic acid, sulfate, or glutathione accumulate to a greater extent in the bile than compounds which are more slowly metabolized. Residue analyses done on extracts of whole undisseminated fish should be interpreted with caution. The microsomal mixed-function oxidases in several species of fish can be induced by specific polycyclic aromatic hydrocarbons or by exposure of the fish to crude oil. Induction of these enzymes can result in quantitative and qualitative differences in the metabolic disposition of xenobiotics to which the fish are exposed. (75 Refs)

326. A REVIEW OF THE GENETIC TOXICOLOGY OF CHLORINATED DIBENZO-P-DIOXINS

Watson JB, Huff JE, Lippert N

Environmental Mutagen Information Center, Information Center Complex/Information Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
Mutat. Res. 47:37-41/1-100 1978

Published and unpublished information on the genetic toxicology of chlorinated dibenzo-p-dioxins, particularly 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is reviewed. TCDD may be formed along with other dioxin compounds during manufacture of the herbicide 2,3,5-trichlorophenoxyacetic acid, the fungicide pentachlorophenol, and the germicide hexachlorophene. Research on dioxins as potential mutagens was initiated because of their structural similarity to acridines, a class of known intercalating agents. Only four dioxins have been evaluated for mutagenicity: the di-, tetra-, and octachlorinated derivatives and the unsubstituted dibenzo-p-dioxin. The results of these evaluations are inconclusive. Experiments with mice suggest that TCDD may be a hepatocarcinogen because of its specific cytological effect on proliferating hepatocytes. However, the cytological effects produced by TCDD in the liver of animals may be due to the toxic effects of the short-term high dose levels administered and to the fact that TCDD tends to concentrate in the liver. The positive mutagenic and cytological effects reported with the few dioxin isomers examined seem to depend on the position of chlorine substitution, with TCDD being the most active form. Preliminary results of cytogenetic analyses of maternal blood and abortive fetal tissue from persons exposed to TCDD in Seveso, Italy (accidental release from a chemical plant), are inconclusive. However, workers exposed to the herbicides Klorinol and Buvinol, which may have varying levels of TCDD contamination, had a higher incidence of chromatid breaks in peripheral lymphocytes than nonexposed individuals. The conflicting results of mutagenicity tests with dioxins could be due to solubility problems, treatment protocols, sample purity, and the extremes in toxicity of these compounds. (70 Refs)

D.

GENERAL

327. DIOXIN

Tuberies FH

Sci. Am. 254:21-29-53 1986

Keywords (MeSH): Acne/chemically induced; Animal; Carcinogens; Dioxins/metabolism/*toxicity; Environmental Pollutants/toxicity; Human; Lethal Dose 50; Sarcoma/chemically induced; Soft Tissue Neoplasms/chemically induced; Tetrachlorodibenzodioxin/metabolism/toxicity.

328. LABORATORY AND HUMAN STUDIES ON POLYCHLORINATED BIPHENYLS (PCBS) AND RELATED COMPOUNDS

Kimberly RD

Center for Disease Control, U.S. Department of Health and Human Services, Atlanta, GA 30333
Environ. Health Perspect. 59:99-106 1985

Similar qualitative toxic effects have been observed in animals for a class of halogenated aromatic compounds, which include the halogenated biphenyls, naphthalenes, dibenzodioxins, and dibenzofurans. All of these compounds are lipid soluble and persist in the environment and in mammals. The polychlorinated biphenyls (PCBs) are the most persistent. They are predominantly stored in fatty tissue; they pass the placenta and are excreted in milk. Some isomers of the halogenated biphenyls are more toxic than others. With some exceptions, the more toxic isomers are retained longer in tissues and are also the carcinogenic components of the mixture. Most of these chemicals seem to be promoters of carcinogenesis in animals rather than initiators. An array of toxic effects in laboratory animals has been ascribed to these compounds and numerous reviews summarizing this information are available. Less information is available on the human health effects of environmental and occupational exposure. Results of recent studies in animals to further elucidate the effects of these chemicals are presented, and results from some human studies conducted in the United States are reviewed. (77 Refs)

128. EXTRAPOLATION OF THE EVIDENCE ON TERATOGENICITY OF CHEMICALS BETWEEN HUMANS AND EXPERIMENTAL ANIMALS: CHEMICALS OTHER THAN DRUGS

Hymenocha K. Vozni P.

Journal of Occupational Health, Helsinki, Finland

Teratogenesis: Carcinog. Mutagen. 5(4):251-316, 1987.

Epidemiologic literature regarding the possible association between malformations and 23 exposures or occupations (other than pharmaceutical products) was analysed. The qualitative level of scientific evidence was classified into four categories: high (ethanol, methylmercury, PCBs, laboratory work), limited (anesthetic gases, carbon monoxide), low (flunitrazepam, LSD, nitrous oxide, smelter work, tobacco), and inadequate (all other exposures). Human data for exposures belonging to categories 'high' and 'limited' were quantitatively compared to results of animal teratogenicity tests of the relevant chemicals. Ethanol, methylmercury, and PCBs have caused malformations in experimental animals, and the effective doses have ranged from 0.2 to 8.0 times the effective human doses. Ethanol and PCBs caused similar types of lesions in some animal species as have been observed in humans. (60 Refs)

530. POLYCHLORINATED BIPHENYLS AND DIBENZOPURANS IN PATIENTS WITH YUSHO AND THEIR TOXICOLOGICAL SIGNIFICANCE: A REVIEW

Manda Y. Yutarnya *et al.*

Daichi College of Pharmaceutical Science, Fukuoka, Japan

Prog. Clin. Biol. Res. 137: 51-68, 1984

The rice oil was ingested by the patients with Yusho and their blood, liver, and adipose tissue were analyzed for individual congeners of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs). The individual congeners identified were examined for accumulation in the liver of monkeys and rats, inducing activities of benzo[a]pyrene 3-hydroxylase, benzphetamine demethylase, and D-T-diaphorase in rats, and gravimetric changes of the thymus and liver in rats. Among the six PCB congeners detected in Yusho patients, 2,3,4,5,3',4'-hexa-CB seems to be the compound most related to Yusho, judging from its strong enzyme-inducing activities in the liver and the thymus atrophy and liver hypertrophy caused by feeding it to rats. PCDF congeners identified in the patients' tissues showed a stronger toxicity in rats than these PCBs, exhibiting stronger enzyme induction activities and gravimetric changes of the tissues. These PCDF congeners, especially 2,3,4,7,8-penta-CDF, were also very accumulative in the liver. Therefore, they are considered as the most important etiologic agents for the current symptoms and signs of Yusho patients.

351. RISK-BENEFIT ASSESSMENT IN IMMUNOTOXICOLOGY

The applicability of immunotoxins as drugs is self-evident. The immunotoxin is a chemical conjugate of an antibody and a cytotoxic drug. The antibody targets the drug to a specific cell, and the cytotoxic drug kills the cell. The immunotoxin is a very effective drug, and it is used in the treatment of cancer. The immunotoxin is a very effective drug, and it is used in the treatment of cancer.

and function or T-cell number and function at the level of antigen uptake, processing and immune response. The process of risk-benefit assessment in immunotoxicology is a large, multidimensional process which seeks to measure the probability and severity of unwanted and adverse immune modulation(s) produced by low levels of chemicals in man. The determined levels of risk to man for any given chemical will fluctuate, depending on the frequency with which new evidence for its toxic effects becomes available. Although benefits derived from the use of a chemical can often be recognized and enumerated with a high level of precision, a qualitative, and particularly a quantitative, assessment of its risks based on available immunotoxicological data is not possible at present. Considerable variation is noted in nearly all known factors involved in risk assessment. Presently, the primary concern of immunotoxicological studies is the development in animals of a battery of *in vivo* and *in vitro* assays which can be used as sensitive indicators of exposure to, or the effects of, a chemical at dose levels comparable with those to which the human population is actually being exposed. The ultimate value of any data derived from animal studies—determining the degree to which risk is considered acceptable under a given set of circumstances, however, will depend upon the qualitative and quantitative accuracy of its extrapolation to man. In designing such experiments, it is necessary to consider that a wide variety of environmental, physiological, and pathological conditions can also influence the immune system. In particular, the hormones of the adeno-hypophysis may exert a direct or indirect effect on the structure and function of the lymphoid organs and on the phagocytic activity of macrophages. Other factors, including the potential immunoregulatory effect of alpha-fetoprotein and malnutrition, should also be taken into consideration when conducting immunotoxicity tests. (23 Refs)

332. ENVIRONMENTAL REACTIONS TO CHEMICAL, PHYSICAL, AND BIOLOGIC AGENTS (87 Refs)

Taylor D. Parrish J.C. Blank III

J. Am. Acad. Dermatol. 11:5 Pt 2:1907-19 1984

Keywords: (MeSH): Animals; Carcinogens; Environmental/adverse effects; Dermatitis; Contact/etiology; Discoloration/adverse effects; Disease Susceptibility; Environmental Pollutants; *adverse effects/analysis; Guinea Pigs; Human; Infants; Newborn; Kinetics; Light/adverse effects; Mice; Occupational Dermatitis/occurrence; Review; Skin Absorption; Skin Diseases; *chemically induced; Skin Neoplasms/chemically induced; Ultraviolet Rays/adverse effects.

133. POLYCHLORINATED DIBENZOFURANS (PCDF): ENVIRONMENTAL AND TOXICOLOGICAL ASPECTS (125 Refs)

June 14

Ann. Ent. Soc. Amer. 2002 95:127-47 (2004)

Key words: (MeSH): Animal, Benzofurans, analysis, metabolism, poisoning, toxicity, Carcinogens, Chlorophenols, toxicity, English Abstract, Environmental Pollutants, Food Contamination, Guinea Pigs, Herbicides, toxicity, Hexachlorocyclopentadiene, Oils, Polychlorobiphenyls, Review, Teratogens, Tissue Distribution.

3.5. THE EFFECT OF UNCERTAINTY IN POLYMERIZATION KINETIC PREDICTION

levels present in the final food product, as well as animal toxicology studies to determine the potential untoward effects of these residues. Extrapolation of residue levels or toxic effects produced by the administration of a chemical in one species to predict residue levels or toxicology that may be produced by lower doses in the same species or by equivalent or lower doses in another species is associated inextricably with knowledge of the pharmacokinetics of the chemical. There are several factors that may influence the pharmacokinetics of xenobiotics which, if left unconsidered, may introduce uncertainty in the predictions of toxicity following any chemical exposure. Two of these factors are dose level and species differences. Because initial toxicity studies are designed to characterize the type of toxicity and to elucidate target organ effects the use of high doses is the accepted practice. However, because biotransformation and excretion of many chemicals are capacity-limited processes, extrapolation of toxicity to lower doses without adequate pharmacokinetic information at those lower dose levels may result in overestimation of predicted toxicity. Furthermore, determination of tissue residue levels following administration of doses that saturate these processes may result in an overestimation of residues remaining following lower doses. Finally, extrapolation of toxicity results across species may result in an over or underestimation of hazard without adequate information on the pharmacokinetics of the chemical in both species. (15 Refs)

335. THRESHOLDS IN CHEMICAL CARCINOGENESIS

Van Duuren BL, Banerjee S

Lab. of Organic Chemistry and Carcinogenesis, Inst. of Environmental Medicine, New York Univ. Medical Center, Cancer and the Environment: Possible Mechanisms of Thresholds for Carcinogens and Other Toxic Substances, New York: Mary Ann Liebert, Inc., p. 85-106 (1981)

The controversial subject of thresholds in chemical carcinogenesis is discussed in an effort to review the various viewpoints that have emerged from the literature and to bring out the complexity of the problems of qualitative and quantitative risk assessment. The threshold concept for chemical carcinogens states that there is a dose or a narrow dose range for a given chemical carcinogen below which cancer does not arise. The single-event (no-threshold) concept, by sharp contrast, implies that one molecule of a chemical carcinogen can interact with a critical receptor in one cell, resulting in an irreversible genetic change that can or will lead to cancer. Test doses used in animal bioassays are at times excessive and not consistent with the definition of the maximum tolerated dose. Many bioassays are carried out at only one or two doses so that reliable dose-response curves, and hence a threshold, cannot be established. In addition, even the lowest doses used in laboratory animal bioassays are frequently several orders of magnitude higher than those encountered in the environment. The involvement of metabolic intermediates in the evaluation of carcinogenicity findings is discussed. Some of those studies in which changes in metabolic pathways and other pharmacokinetic aspects have been shown to occur with change in dose and route of administration are dealt with. The metabolic processes involved, covalent interaction with biomacromolecules, and enzymatic detoxification of xenobiotics are saturable processes, i.e., above certain dose levels, dose-dependent linear pharmacokinetics become non-linear. These important types of pharmacokinetic studies, or lack thereof, are frequently overlooked in the evaluation of carcinogenicity bioassays and in risk assessment. The subject of human risk assessment is briefly referred to in relation to the matter of thresholds. (53 Refs)

336. HUMAN AND ENVIRONMENTAL RISKS OF CHLORINATED DIOXINS AND RELATED COMPOUNDS

Tucker RE, Young AL, Gray AP

Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, Tucker RE, Young AL, Gray AP (Eds.), Plenum Press, New York, 1983

337. PHENOXY ACIDS: A BRIEF REVIEW OF THE POSSIBLE HYGIENIC EFFECTS

Edling C

Yrkemedicinska kliniken, Regionalsjukhuset, Linköping, Sweden, Lakertidningen, 78(34):2861-2862 (1981)

Recent studies on the hygienic effects of phenoxy acids used as pesticides are reviewed. 2,3,7,8-Tetrachloro-dibenzo-p-dioxin, occurring in a small amount as impurity in (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), was found to be a carcinogen and possibly a promoter in experimental animals. Small quantities of other dioxins, eg, tetrachloro-p-dioxin, were found in (2,4-dichlorophenoxy)acetic acid (2,4-D). Increased incidence of tumors and malformations due to 2,4,5-T was found in mice, and increased incidence of lymphosarcoma and tumors of endocrine organs was found in rats treated with 2,4-D. The mutagenicity tests of the phenoxy acids and dioxins yielded contradictory results. No carcinogenic effect of 2,4-D and 2,4,5-T was demonstrated in some animal experiments. Epidemiological studies showed a link between soft-tissue sarcoma and lymphosarcoma and exposure to phenoxy acids. Other epidemiological studies showed no relationship. Yet, a link between cancer and exposure to 2,4,5-T seems to exist in the light of experimental and epidemiological data. (10 Refs)

338. LONG-TERM HAZARDS OF POLYCHLORINATED DIBENZODIOXINS AND POLYCHLORINATED DIBENZOFURANS

Hall JE, Moore JA, Saracci R, Tomatis L

Natl. Toxicology Program, P.O. Box 12233, Research Triangle Park, NC, 27709, Environ Health Perspect, 46:221-260 (1980)

An ad hoc Working Group of the National Institute of Environmental Health Sciences/International Agency for Research on Cancer met to discuss hazards associated with exposure to chlorinated dibenzo-p-dioxins (PCDD) and chlorinated dibenzofurans (PCDF). The collective views and opinions are reported, with epidemiological studies comprising the bulk of the report. The toxicological aspects of PCDD and PCDF in animals and humans, and episodes of human exposure to these compounds are discussed. The data indicate that a long-term hazard associated with these compounds might be increased cancer rates. The Working Group recommended that a system be developed for an international exchange of information and research coordination on the health effects of PCDD and PCDF. Special attention should be given to developing sensitive and reliable measurements of body burden, common protocols for clinical examinations, and an international registry of exposed persons. (135 Refs)

339. PERSISTENT POISONS: CHEMICAL POLLUTANTS IN THE ENVIRONMENT

Schroeder M

No affiliation given

Persistent Poisons: Chemical Pollutants in the Environment, New York: The New York Academy of Sciences, 67 pp., 1979

Halogenated aromatic hydrocarbons and their effects and control are described. In an introductory part, some recent accidents with chemical pollutants (dioxin at Seveso, Italy; polychlorinated biphenyls (PCBs) in Michigan; and polybrominated biphenyls (PBBs) in Yusha, Japan) are discussed.

Other parts include discussions on the halogenated aromatic hydrocarbons (DDT and other organochlorine pesticides, PCBs, dioxins and dibenzofurans, PBBs, and other pollutants), health effects (general symptoms, the search for under-

lying metabolic disturbances, neurologic effects, immunologic effects, carcinogenicity, and reproductive effects), and taking action (decontamination, identifying the problems, and avoiding further contamination). (no Refs)

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